

1970

The ultrastructure of the nuclear division of *Basidiobolus ranarum* Eidam

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70-18,912

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THE ULTRASTRUCTURE OF THE NUCLEAR DIVISION OF
BASIDIOBOLUS RANARUM EIDAM.

Iowa State University, Ph.D., 1970
Biology-Genetics

University Microfilms, A XEROX Company, Ann Arbor, Michigan

THE ULTRASTRUCTURE OF THE NUCLEAR DIVISION
OF BASIDIOBOLUS RANARUM EIDAM

by

Nai-Chau Sun

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Cell Biology
and Genetics

Approved:

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1970

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I. INTRODUCTION

There have been numerous debates about the manner of somatic nuclear division in fungi. The mitotic apparatus and the sequence of events cannot be easily resolved with light optics. A number of workers have viewed fungal nuclear division as "classic" mitosis (Somers, Wagner and Hsu, 1960; Ward and Ciurysek, 1962; Robinow, 1963; Hartman, 1964) while others have claimed that an amitotic mechanism is involved (Bakerspiegel, 1959a, b, and c; Turian and Cantino, 1960; Slifkin, 1967). A third suggestion is that the nucleus elongates and then constricts under the influence of the spindle apparatus (Girbardt, 1962; Aist, 1969).

Extensive fine structure studies of somatic nuclear division in fungi have been made only in few species. In each study synchrony of division was achieved by one means or another. Synchronously dividing yeast cells were studied by Robinow and Marak (1966); synchronous nuclear division in plasmodia of myxomycetes by Guttess, Guttess, and Ellis (1968), and Aldrich (1969), and in the process of sporangium formation in several phycomycetes by Ichida and Fuller (1968), and Lessie and Lovett (1968).

Random scanning of the electron microscope screen is an inefficient and frustrating method of studying mitosis in all asynchronously dividing tissue, especially in fungi,

where small nuclei and almost insignificant chromosomes greatly complicate the problem. The success of such a study depends on the ability to preselect cells at various known stages and to orient these cells so that they can be sectioned in predetermined planes. The ability to section a dividing nucleus at a known stage in a known plane is essential but difficult in most fungi. The open-face embedding technique, permitting the preselection of cells under light optics has provided extremely instructive information in the analysis and description of mitosis (Robbins and Gonatas, 1964b; Barnicot and Huxley, 1965), and nuclear organizers (Hsu, Brinkley, and Arrighi, 1967). The present application of this technique to fungi has yielded new information on nuclear division.

The first published cytological investigation of the fungus Basidiobolus ranarum, a member of the family Entomorphothoraceae in the Zygomycetes, was done by Raciborski (1896) who published some drawings of dividing nuclei. Even though later detailed studies of nuclear division in this species with light optics were well-documented as classic mitosis (Robinow, 1963; Robinow and Bakerspiegel, 1965; Brown and Bertke, 1969), this matter did not appear to be resolved. Robinow's descriptions of the importance of the nucleolus in the formation of spindle fibers, the absence of the nuclear envelope throughout most of the division, the

persistence of the nucleolus, and the lack of centrioles were questioned in this laboratory.

Basidiobolus ranarum has a large single nucleus in each cell, and therefore eliminates problems with respect to determining stage of division before processing. The purpose of this present work is to reinvestigate the nuclear division in this species employing electron microscopy and a modification of the open-face technique (Bloom, 1960; Brinkley, Murphy, and Richardson, 1967).

II. LITERATURE REVIEW

A. The Life Cycle of Basidiobolus ranarum

Basidiobolus ranarum Eidam was described by Eidam (1887) as a saprophyte on the excrement of frogs. An extensive discussion of the morphology and taxonomy of the genus was given by Eidam and soon afterward Thaxter (1888) published notes of growing this species on frog excrement. The forceful ejection of sporangia from the sporangiophore was taken advantage of by Olive (1907) to obtain a pure culture. He allowed sporangia from cultures grown on frog excrement to be "shot" onto a small cube of sterilized bread.

The mycelium of Basidiobolus ranarum develops profusely on the frog excrement in two to three days. Under normal conditions it consists of ramose hyphae composed of uninucleate cells. Multinucleate cells are found under conditions of aging or poor nutritions. The fungus grows well on various artificial media but now it is usually maintained on yeast extract agar (Robinow, 1963). Its unusual growth pattern was described by Raciborski (1896) as "stepwise growth", i.e., the protoplast keeps moving forward leaving a long chain of empty cells.

In asexual reproduction, each cell grows into a slender, erect sporangiophore, which becomes inflated or swollen at its raised end. A terminal bud is formed into which the

nucleus and considerable cytoplasm migrate. The bud gradually enlarges to form a pear-shaped, uninucleate sporangium (conidium). The sporangium is then forcefully discharged when the sporangiophore splits and releases pressure built up by the continuous absorption of water by the sporangiophore from the mycelium.

Later the sporangia may be eaten by beetles that in turn may be eaten by frogs. In the intestinal tract of the frog the sporangium undergoes further development; three nuclear divisions occur forming eight thin-walled non-motile sporangiospores which escape when the sporangial wall ruptures. Normal mycelia develop from these spores after excretion.

Under conditions of poor nourishment, the mycelium forms zygospores by the production of juxtaposed protuberances, partial solution of the separating walls, and movement of the protoplasm and nucleus from one of the gametes into the other. The fusion cell lays down a thick, layered wall. After a rest period the zygote may germinate with a germ tube into a mycelium. A meiotic division presumably takes place before germination.

B. Fungal Nuclear Division

1. Light microscopy

It is generally recognized that the study of fungal cytology with light optics is handicapped by the small size of fungal nuclei (usually about 2-3 μ m), tiny chromosomes, and the low affinity of somatic fungal chromosomes for the more usual chromosomal stains. As a result, the literature concerning light microscopic studies of fungal nuclear division is confusing and has resulted in conflicting and erroneous opinions. A pragmatic approach arbitrarily places the various putative mechanisms, described to date, into the following categories:

a. Amitosis, or Mucor-type nuclear division Many fungi have been described as having a dividing nucleus that elongates and pinches into two daughter nuclei (Table 1).

b. Intranuclear division with centrioles, spindle fibers and distinct chromosomes This type of division was well-illustrated in Macrophomina phaseoli (Knox-Davis, 1968). The entire process occurred within an intact nuclear envelope. At prophase the individual chromosomes could be recognized. At metaphase a top-shaped spindle with spindle fibers and two terminal centrioles developed. The chromosomes became attached to the spindle at different points.

Table 1. Fungi with a Mucor-type Nuclear division

Fungus	Author	Year
<u>Mucor hiemalis</u> ; <u>M. fragilis</u> ; <u>Phycomyces</u>	Robinow	1957
<u>Blastomyces dermatitidis</u>	Bakerspiegel	1957
<u>Endogone sphagnophila</u>	"	1958
<u>Schizophyllum commune</u>	"	1959a
<u>Neurospora crassa</u>	"	1959b
<u>Gelasinospora tetrasperma</u>	"	1959c
<u>Candida</u> spp.	Widra	1959
<u>Saprolegnia</u>	Bakerspiegel	1960a
<u>Scorulariopsis</u>	"	1960b
<u>Ophiostoma fimbriata</u>	"	1961
<u>Lipomyces</u>	Robinow	1961
<u>Ceratobasidium praticolum</u>	Thyagarajan and Naylor	1963
<u>Saccharomyces</u> , <u>Schizosaccharomyces pombe</u>	Yoneda	1963
<u>Lipomyces starkeyi</u> , <u>Torula utilis</u> , and <u>Torula rubra</u>		
<u>Candida</u> spp.	Bakerspiegel	1964

Anaphase was characterized by the separation of chromatids to either pole, and, in telophase, the interzonal fibers between each chromatid disappeared; the two nuclei reformed.

Aist (1969) reported similar types of division in Ceratocystis fagacearum and Fusarium oxysporum. Girbardt (1962) established the presence of this type of division in Polystictus but failed to locate centrioles. The fission yeast, Saccharomyces versatilis, shared essentially the same kind of spindle and distinct chromosomes as in Polystictus (Robinow and Bakerspiegel, 1965).

c. Classic mitosis Using light microscopy, Basidiobolus ranarum (Robinow, 1963) was reported to have a typical mitosis similar to that of higher plants. The outline of the nucleolus "blurred out" at the beginning of prophase while some of its material appeared to contribute to spindle fibers. The rest of the nucleolar material developed into "end plates". At metaphase closely packed, tiny chromosomes formed a "Saturn's ring" at the equator of the spindle. At anaphase the chromatids separated from each other and moved into the end plates. The end plates finally broke down into granules and droplets in telophase. The two nuclei reformed.

Classic mitosis was claimed for Neurospora crassa (Somers, Wagner, and Hsu, 1960; Ward and Ciurysek, 1962) and for Alternaria tenuis (Hartman, 1964); however, evidence for spindle fibers was not convincingly presented in any of these studies.

d. Weijer-type nuclear division Weijer and his associates presented a highly controversial and complicated interpretation of somatic nuclear division in Neurospora (Dowding and Weijer, 1960, 1962; Weijer, Koopmans, and Weijer, 1963, 1965; Weijer and MacDonald, 1965), and Aspergillus (Weijer and Weisberg, 1966). During division, the chromosomes physically become linked together end-to-end into a compound chromosome which assumes a "ring" configuration in the middle of the spindle with a centriole at either pole. The compound chromosome comes to lie in a plane parallel to the spindle axis. Division is achieved when the double rings or double filaments split longitudinally and slide apart. During this separation stage, daughter chromosomes are maximally condensed and recognizable.

C. L. Wilson and his students studied a wide range of plant pathogenic fungi: Ceratocystis fagacearum (Aist and Wilson, 1967), Ceratocystis coerulescens, Fusarium oxysporum, Alternaria cucumerina, Collectotrichum gossypii, Formes annosus, Lenzites saepiaria, Rhizoctonia solani, Sclerotium rolfsii, Thielaviopsis basicola, and Verticillium albo-atrum (Brushaber, Wilson, and Aist, 1967). Their interpretation of nuclear division differed from that of Weijer's only in that the spindle was formed between separating chromatids. However, a rather thorough study by Heale, Gafoor, and Rajasinghan (1968) in Verticillium albo-atrum suggests that

the spindle was formed parallel to the plane of the chromosomes.

2. Electron microscopy

The use of electron microscopy by many fungal cytologists has already provided unequivocal evidence of the existence of the mitotic apparatus in certain fungi and will eventually help to understand nuclear divisions in all fungi.

Moore (1964) identified the Mucor model of fungal nuclear division when he examined dividing nuclei of Cordyceps militaris. He was unable to detect either centrioles or an intranuclear spindle during division. He coined the term "karyochorisis" for the Mucor-type nuclear division.

During a study of Albugo candida infecting radish, Berlin (1964) found centrioles at both poles of dividing nuclei. These dividing nuclei were characterized by intact nuclear envelopes and intranuclear spindles. Allomyces macrogynus (Robinow and Bakerspiegel, 1965) and Blastocladiella emersonii (Lessie and Lovett, 1968), both reported to have amitotic divisions in light microscopic studies, were also shown to have intranuclear divisions. Chromosomes were not observed in these species. Ichida and Fuller (1968) demonstrated chromosomes during the intranuclear division of the water mold Catenaria anguillulae.

In baker's yeast centriolar plaques attached to the nuclear envelope assumed the function of the centriole in serving as organizing centers of the aster and the intranuclear spindle. However, Moor (1966) employing the freeze-etching technique, failed to demonstrate the presence of asters in yeasts, even though intranuclear spindles were seen. This type of mitosis with centriolar plaques was also demonstrated in Pustularia cupularis (Schrantz, 1967).

Other types of modified centrioles were also reported. For example, Armillaria melee (Motta, 1967) had densely stained, centriole-like bodies located in the cytoplasm adjacent to either pole of the mitotic nucleus which was intact throughout the process. In Polystictus versicolor, Girbardt (Burnett, 1968) described strongly basophilic, atypical centrioles to which spindle fibers were attached.

Two types of mitoses, the myxamoebal mitosis and the plasmodial mitosis were described by Aldrich (1969) in Physarum flavicomum, a slime mold. The former type was characterized by an amphiastral spindle organized by centrioles in the cytoplasm and the disruption of the nuclear envelope during prometaphase, while in the latter an intranuclear spindle formed in the absence of the centriole and nuclear envelope remained intact throughout division. Guttess, Guttess, and Ellis (1968) described the latter type of mitosis in plasmodia of P. polycephalum.

C. Centriole

Several recent review articles have extensively covered electron microscopic studies of the centriole and basal body in animals (Stubblefield and Brinkley, 1967; de Harven, 1968), in plants (Newcomb, 1969), and in fungi (Bracker, 1967).

The centriole is found in the cytoplasm of almost all animals (Mazia, 1961), in some algae (Berkaloff, 1963; Johnson and Porter, 1968), in fungi (Berlin and Bowen, 1964; Renaud and Swift, 1964; Lessie and Lovett, 1968) in some gymnosperms (Mizukami and Gall, 1966), but not at all in angiosperms. Microtubules have been reported in association with one of the centriolar triplet tubules (Gall, 1961), with the accessory structures of the centriole, the satellites (Robbins and Gonatas, 1964b; de Thé, 1964), with the triplet bases of centrioles (Stubblefield and Brinkley, 1967), and with a pad appressed to the centriole (Dippel, 1968). Whether or not these are all usual and normal associations is not clear, however, it is apparent that the centriole serves as an organizing center for the production of microtubules (Inoue and Sato, 1967).

In 1956, de Harven and Bernhard were the first to describe the centriole using electron microscopy, and their description has been subsequently confirmed by Yamada (1958), Bessis and Breton-Gorius (1957), Amano (1957), and many

others. Berlin and Bowen (1964) were the first to find centrioles in fungi.

Basically the centriole is a cylindrical body about 0.25 μm in diameter, 0.5-0.7 μm in length. Its outer wall consists of nine evenly spaced triplet microtubules running parallel to its long axis. Each triplet consists of a row of three appressed microtubules. The nine triplets are so arranged that each row of microtubules makes an angle of about 40° with the tangent to the cylinder. The microtubules of each triplet, embedded in a common matrix, are designated as subfiber A, B, and C with A the innermost unit. From subfibers A, there are two diverging arms, one directed outward to connect with subfiber C of the next triplet, the other arm directed inward along a radius, the foot. Bounded peripherally by the matrix of the centriole wall, and internally by the foot, is the triplet base which appears to be a linear structure running parallel to the triplet microtubules. Within the centriole lumen are a small 60 nm vesicle and a large helix. At one end of the centriole a cartwheel structure that consists of 9 radial spokes in the center can be visible (Stubblefield and Brinkley, 1967).

Modifications of the standard centriolar architecture have been reported. In Sciara two types of centrioles, differing from the standard type, were described by Philips (1967); the first is composed of nine doublet tubules rather

than the usual nine triplet tubules, and the second type is the giant centriole with 60-90 singlet tubules arranged in an oval. A single axial filament in the proximal three-fourths of the centriole was reported in Albugo candida (Berlin and Bowen, 1964), and in Allomyces (Robinow and Bakerspiegel, 1965).

Stubblefield and Brinkley (1967) induced centriole-basal body transformation in cultured fibroblasts which normally lack flagella. This experiment supported the old idea of the interconvertability of these two organelles.

The manner in which centrioles are perpetuated has been elusive because they are relatively small and few per cell. Stubblefield and Brinkley (1967) claimed that they were able to see centrioles only in 6% of their sections and most of them were not aligned properly. However, basal bodies do occur in a great number of ciliates where they replicate prior to completion of cell division, a fact which could lead to the understanding of proliferation of the centriole. Gall (1961) observed the formation of a group of eight daughter procentrioles in a two-dimensional array at right angles about the axis of a mature centriole during maturation of multi-flagellated sperm in Viviparus. This is in agreement with the budding theory of centriole replication proposed by Bernhard and de Harven (1960), although the ratio of pre-existing to nascent centrioles is 1:1. Recently

Dippel (1968) clearly showed how the budding of a daughter basal body is accomplished step by step from the mature basal body in Paramecium. At first, a flat disk of dense, fibrous material appears close to the basal body. Then the subsequent formation of a ring of nine singlet microtubules, one by one, is followed by a second and third round of tubule proliferation to form the nine-triplet procentriole. This does not support Lwoff's hypothesis (1950) that a basal body is always derived from a preexisting one directly, tubule for tubule. However, the possibility of genetic continuity between basal bodies was supported by the evidence for DNA in the basal body (Smith-Sonneborn and Plaut, 1967).

This manner of perpetuation of the basal body was suggested by Dippel (1968) to have general application in other organisms, and indeed a similar picture was reported in Tetrahymena (Allen, 1969). Additional support comes from the observation of nascent procentrioles around small "clouds" of dense material which bear a superficial resemblance to the disc (Dirksen and Crocker, 1965).

D. Open-face Embedding Technique

Borysko and Sapranaukas (1954) should be credited with the introduction of the open-face or in situ embedding technique to electron microscopy. This permits the embedding of whole colonies of cells in plastic without removing them

from the substrate, usually the surface of a glass cover slip. Cells may be continuously observed with a phase-contrast microscope and collected at any stage desired for electron microscopy. By means of this novel technique, not only can the effects of fixation be studied, but those of dehydration, impregnation, and embedding. For preparing ultrathin sections of smears or squashes Gay (1955) independently developed a technique to cover the portion of the smear with a gelatin capsule containing partially polymerized methacrylate which was later separated from the glass surface due to the lack of adhesion between the two. Howatson and Almeida (1958) modified the above technique for embedding tissue culture cells growing as a thin layer on the surface of a slide or a Petri dish. The cells were fixed, dehydrated and infiltrated with methacrylate without removal from the glass surface. After polymerization the capsule was detached by chilling the glass surface with a block of dry ice. When Latta (1959) found it was difficult to separate a large polymerized methacrylate layer from the bottom of the Petri dish, he placed small rings on solid methacrylate around the selected cell colonies. These wells were filled with fresh monomer and catalyst.

Frequently, when the plastic was separated from the glass surface, the cells were found either to be damaged or were still attached to the glass surface. To prevent

this, either the culture could be made on mica or a pale grey coating of carbon could be deposited on the glass slide (Bloom, 1960).

Epoxy resin was found to be very difficult to separate from the glass on which the cells were grown. To provide a fracture plane which permits the easy separation of epoxy resin and the glass surface, the use of reconstituted rat-tail collagen was recommended by Heyner (1963), a carbon film was deposited on the slide by Robbins and Gonatas (1964a) mica was substituted for the glass substrate (Persijn and Scherft, 1965), and the slide was coated with a layer of Formvar by Stobb, Amy, Wertz, Fauconnier, and Bessis (1966).

Sutton (1965) found that plunging hot polymerized Araldite or Maraglass into an ice water bath resulted in a separation between the plastic and a glass surface. When tissue culture cells were grown in a plastic container, the propylene oxide step in the infiltration procedure had to be replaced by a soak in water-miscible resin (hydroxypropyl methacrylate) which mixed well with both alcohol and Epon 812. The Epon plate could be separated from the container by cooling the container on dry ice (Brinkley, Murphy, and Richardson, 1967).

III. MATERIALS AND METHODS

A. Phase Contrast Microscopy

Basidiobolus ranarum Eidam was obtained from Dr. C. L. Wilson, Plant Pathology Department, University of Arkansas, Fayetteville. This fungus was grown and maintained at room temperature (24°C) on plates of yeast extract glucose agar medium (0.5% yeast extract, 2% glucose, 2% starch, and 2% agar) with a final pH 6.2. Transfers were made quickly.

Standard microscope slides, cleaned and sterilized with acetone-wet Kimwipes, were dipped in hot, molten yeast extract glucose agar (60°C), drained for a few seconds, and placed in the 60°C incubator to dry the agar film rapidly. Contamination was never a problem. A trace of inoculum from a healthy culture was placed in the center of the agar-covered slide. The inoculated slide was inverted over a plastic dish (3 inches x 1 inch x 1 inch, the wall 1/8 inch thick) half-filled with distilled water. The dish and slide were transferred to a moist chamber modified from an ordinary desiccator with the bottom filled with distilled water. The fungus was allowed to grow for 24 hours at room temperature. When suitable growth was obtained, the inoculated slide was taken out from the moist chamber, and the back side wiped clean. A drop of liquid yeast extract glucose medium and No. 1 cover

slip were put over the growing mycelium. When the uncovered part of the agar film dried out, the edges of the cover slip were sealed with dental wax. At this point the mycelium was ready for observation under Leitz Ortholux phase contrast microscope equipped with a Leitz Orthomat 35 mm automatic camera.

B. Electron Microscopy

A clean, transparent 3 inches x 5 inches mica sheet (Persijn and Scherft, 1965; obtained from McMaster Corp., Chicago, Illinois), was cut into five 3 inches x 1 inch mica slides. Each slide was thoroughly cleaned and sterilized with acetone-wet Kimwipes and dipped into fresh, hot, molten agar medium. The agar medium was stored in a capped bottle of a size to accept a slide in a 60°C incubator. Only fresh agar, less than two hours in the incubator, gave a film strong enough to sustain the subsequent changes of solutions without being washed away. The starch component of the medium provided strength for the agar film. Without it, the film would separate from the mica slide after a few minutes in the fixative. The agar-coated slide was dried in the 60°C incubator for 1-2 hours. Four agar blocks (10 x 2 x 2 mm) were cut from the periphery of a healthy culture and

placed in a row near the center of the mica slide. The inoculated slide was placed up-side-down on a plastic dish half-filled with distilled water and together the dish and slide were transferred to a modified moist chamber at room temperature. The purpose of this manipulation was to facilitate the mycelial growth, yet keep the agar film on the back side of the slide dry enough so it would not come off in subsequent washes. An average of 25-30 mitotic figures were counted in the mycelium in each block after 15-17 hours growth under this condition.

For fixation, a slide with growing mycelia was eased into an aluminum foil boat (3 1/8 inches x 1 1/8 inches x 1/2 inch) contained 5 ml of 6% glutaraldehyde in 0.1 M Sorenson's phosphate buffer at pH 6.2, and an additional 5 ml of fixative was pipetted on afterwards, completely submerging the slide. Thirty-minute fixation in glutaraldehyde was followed by 3 ten-minute washes in the above buffer only. Postfixation in 1% osmium tetroxide in the same buffer was followed by 3 five-minute washes in distilled water. Dehydration consisted of five-minute intervals in 25%, 50%, 75%, 95% ethanol, and 0.5% eosin in 95% ethanol. The eosin stain was used to facilitate the visualization of desired hyphae in subsequent procedures. Three five-minute changes of 100% ethanol completed dehydration. Dehydrated material was passed through 3 five-minute changes of propylene oxide

and in the first round of propylene oxide, the agar inoculum blocks were carefully lifted off leaving the agar film containing mycelia on the slide. The material was then placed in an embedding mixture (modified from Anderson and Ellis, 1965) containing 30 gm of DDSA, 11.4 gm of Araldite 502, 15.1 gm of Epon 812, plus 1.5 ml of DMP-30. After infiltrating overnight, the mica slide was lifted out of the aluminum foil boat with a pair of forceps. Excess Epon-Araldite mixture was removed by a wood stick. The total thickness of slide and plastic should not be greater than that of a mica slide with a cover slip. The mica slide was then transferred to a new aluminum foil boat with one end of the slide resting on a short edge of the boat. Polymerization followed, 12 hours at 35°C, 12 hours at 45°C, and 12 hours to several days at 60°C. When polymerized resin on the back of the slide was peeled off, the slide could be placed under a phase contrast microscope and examined with a high dry objective. Mitotic cells in various stages were photographed or simply sketched in a record book and marked with a dent on polymerized resin over the cell by a sharp bent needle while still on the microscope under low power. The dent was marked with 2% aqueous crystal violet delivered by a sharpened toothpick. The marked resin was cut out with a sharp razor blade under a dissecting microscope to a size of about 1 mm² and peeled from the mica

slide. This was glued with epoxy cement to a dummy block, the marked side down. After the glue was hardened in a 60°C incubator for a period of 3-6 hours, the block was ready to be trimmed into a trapezoid, with the two parallel sides parallel to the long axis of the hypha. Guided by the blue mark, it was a simple matter to trim the block very close to the hypha. After the first 5 μ m of the embedding medium was trimmed away with a glass knife, thin sections were cut with a Rondkin diamond knife, picked up on 100-mesh, carbon - and Formvar-coated grids, and stained with methanol uranyl acetate (15 minutes) (Stempak and Ward, 1964) and lead citrate (15 minutes) (Reynolds, 1963) successively.

Specimens were observed with an RCA EMU 3F or Hitachi 11-C electron microscope operated at 50 Kv.

IV. OBSERVATIONS AND RESULTS

A. Observations on Living Cells with Phase Contrast Microscopy

The elongate hyphal cell (200-400 μm) has a spindle-shaped nucleus (25 x 10 μm) mostly occupied by a large nucleolus 10-15 μm long and 5-7 μm in diameter in the center of the nucleoplasm (Figures 1a and 1b). The peripheral boundary of the bright area surrounding the nucleolus is apparently the interface between nucleus and cytoplasm; however, the nuclear envelope is never visible under phase contrast microscopy.

The nucleolus sometimes contains several nucleolar vacuoles (Robinow, 1963) (Figure 1a). Occasionally the nucleolar vacuoles appear to fuse into one large nucleolar vacuole. Whenever such fusion was seen, the nucleolus subsequently appeared to split into two smaller nucleoli. Despite many observations, nuclei with nucleolar vacuoles were never observed to go into mitosis.

Under the conditions of growth used here, cell division is never synchronous in the tip cells of a given mycelium. Of several thousand tip cells in one mycelium, from 20 to 30 were found in mitosis.

The first sign of prophase is the gradual dispersion of the nucleolus into the nucleoplasm. As prophase

progresses the residue of the nucleolus gradually disappears. By prometaphase, the then spherical nucleus has a distinctive, diffuse, appearance (Figures 1c and 1d). It is not possible to recognize the disruption of the nuclear envelope under light microscopy.

The onset of metaphase is marked by the formation of two separate bodies, one at each pole of the nucleus. At first they are faint and difficult to distinguish but gradually they darken until they appear similar to interphase nucleoli. As they increase in size and density these two bodies emerge as dense, cap-shaped end-plates (Robinow, 1963) narrowly separated on either side of a long bright zone, the spindle (Figures 1e and 1f). Chromosomes are not visible during this stage. When the end plates begin to round up, a dark band of chromosomes, the metaphase plate, appears at the equatorial plate of the spindle (Figures 1g and 1h).

By telophase the continuous expansion of the interzonal spindle has resulted in wide separation of the two end plates which increasingly resemble nucleoli. A slow rotation of the spindle axis from the direction perpendicular to the hyphal long axis to the one parallel with the hyphal long axis has taken place (Figures 1h and 1k). The lighter appearance of the interzonal spindle under phase contrast is lost at this time (Figure 1L). The two daughter nuclei move to opposite

ends of the long cell, however, the cross septum is not formed immediately.

B. Observations with Electron Microscopy

1. Interphase

The interphase nucleus is a large prolate spindle-shaped body, 25 x 10 μm , delineated by a double-membraned nuclear envelope which occasionally exhibits some finger-like invaginations into the nucleoplasm (Figures 2, 3, and 4). The orderly arranged nuclear pores, 70-100 nm in diameter, do not appear to provide a free passageway between the cytoplasm and nucleoplasm. Each appears to contain a plate of rather dense material, the nuclear plug, about 20 nm thick.

The large elliptical nucleolus (10-15 μm long and 5-7 μm in diameter) (Figures 2, 3, 8, and 9) is similar in substructure to nucleoli of other plant cell types (Lafontaine and Chouinard, 1963; Chouinard and Leblond, 1967; Lord and Lafontaine, 1969). At the fine structure level, the nucleolus consists of three distinct structural components: a granular zone made up of dense particles 15-20 nm in diameter; a fibrillar zone intermingled with the granular zone and consisting predominantly of densely packed fibrillar material 6-8 nm in diameter (Figures 2 and 6); and a third zone, the proteinaceous matrix, containing lightly stained

amorphous granules (Figure 6)(Hay, 1968). The proteinaceous matrix also appears identical with the nucleolar vacuole described by Robinow (1963) with the light microscope and by Lafontaine and Chouinard (1963) with the electron microscope. Less dense areas are found in the fibrillar zone and correspond to the DNA-containing lacunae described in nucleoli of Allium cepa (Chouinard, 1966a). Hay (1968) coined the terms "pars granulosa" for the granular zone and "pars fibrosa" for the fibrillar zone.

The nucleoplasm in which the nucleolus is embedded is of only moderate density and is made up of relatively evenly distributed fine fibrils 5 nm in diameter. Five nm granules are also seen which tend to aggregate together and form clusters of granules up to 30 nm in diameter (Figure 4).

Cytoplasmic microtubules 20 nm in diameter, similar to those described by Ledbetter and Porter (1963) in root tips of Phleum and Juniperus are found in the cytoplasm. They are either located peripherally near the plasmalemma or several micrometers away from it (Figure 5), but they tend to be aligned parallel to the long axis of the cell. Their number is less numerous and their distribution more irregular in comparison with cytoplasmic microtubules reported in root tips of Phleum and Juniperus (Ledbetter and Porter, 1963), and radish (Newcomb and Bonnett, 1965). Because microtubules are implicated in cell wall formation and since fungal hyphae

grow mainly by apical extension, studies of cytoplasmic microtubules have been extended to the apical tip of the hypha. As observations are carried out near to the hyphal tip, cross and oblique sections of microtubules become evident (Figure 10). It is not certain whether or not this random network of microtubules in the dome of the hyphal tip plays a role in wall orientation as suggested by Newcomb and Bonnett (1965).

A number of multiple vesicular bodies, similar to those described by Robbins and Gonatas (1964b) in Hela cells are found throughout hyphae (Figure 12). Vesicles, measuring about 80-160 nm in diameter, are found only in the dome and filled with a homogeneous substance (Figure 17).

The cytoplasm contains many ribosomes 20 nm in diameter, tubular rough ER (Figure 5), filamentous mitochondria, and occasionally spherical microbodies (200-400 nm) with very dense amorphous contents in which crystalline-like structures are often embedded (Figure 7).

Contrary to the proposal by Robinow (1963) that the absence of centrioles in B. ranarum is due to the fact that there is no motile stage in the life cycle, extra-nuclear centrioles were evident in the material studied here. The centriole of B. ranarum is a very tiny structure, about 0.14 μ m in both diameter and length. This contrasts with centrioles of other organisms where a diameter and length of

about 0.15 μm and 0.5 μm respectively is typical. Centrioles of B. ranarum occur in small depressions of the nuclear envelope and appear appressed to the outer membrane. Throughout this study of the interphase nucleus and various mitotic stages, only longitudinal and tangential profiles of centrioles were seen. Unfortunately no transverse sections were found despite an arduous search, and so a comprehensive description of this centriole must await further studies. However, it has an architecture showing parallel distribution of microtubules, 20 nm in diameter, embedded in an osmophilic matrix which often masks the tubular wall. A central hub extending almost the complete length of the centriole as observed by Berlin and Bowen (1964) in Albugo and by Robinow and Bakerspiegel (1965) in Allomyces appears to be a feature of this centriole although this was only clearly observed once.

In about a dozen observations of centrioles associated with different interphase nuclei, centrioles were never seen in pairs. In one set of serial sections through a single interphase nucleus a single centriole was seen at one end of the elongate nucleus and in a different section another single centriole was seen near the middle of the nucleus (Figures 8, 9). The typical arrangement of two centrioles perpendicular to each other was seen several times in metaphase and anaphase.

2. Prophase

The earliest indication of the onset of mitosis is the initiation of a series of characteristic morphological changes in the huge nucleolus. Two distinct zones, the pars granulosa and the pars fibrosa, which are evident in the interphase nucleolus, appear much lighter because they disperse, filling up most of the nuclear space (Figure 13). The granular elements of the pars granulosa are still detectable because of their greater density and larger size compared with the nucleoplasmic granules (Figure 14). The dense 20 nm thick nuclear plug of the interphase nucleus appears thinner (5 nm) and of a much more diffuse texture.

In prophase the centrioles are usually found at nearly opposite ends of the elongate spindle-shaped nucleus forming an axis shorter than but roughly parallel to the median long axis of the nucleus. The nuclear envelope frequently is deeply indented around the centriole (Figure 13).

As prophase progresses, increasing numbers of 20 nm microtubules appear in the general vicinity of the poles. These generally lie in planes tangential to the nuclear envelope. Some, but not all of these, radiate from the centriole as a focus. Since the centriole generally lies in a depression of the nuclear envelope, many of these microtubules terminate at or very close to the outer membrane of

the nuclear envelope. Occasionally a microtubule appears to penetrate the envelope apparently through a pore (Figure 17).

The pars granulosa has disappeared from view and its components, the nucleolar granules, now are loosely and uniformly distributed within the domain of nuclear space. The more persistent pars fibrosa apparently now starts to disperse. Small patches of nucleolar fibrils (Figures 16, 17, and 18) can be found separated from the main mass of fibrils. Eventually all nucleolar fibrils disintegrate and intermingle undistinguishably with the components of the nucleoplasm.

At late prophase, accompanied by the progressive breakdown of the pars fibrosa (Figure 21), the nuclear envelope begins to disrupt into small vesicles and short cisternae in the vicinity of the poles (Figures 19, and 20).

3. Prometaphase

Early prometaphase is marked by a complete disorganization of the nuclear envelope into many smooth-surfaced vesicles and short cisternae. These envelope fragments remain roughly in a sphere around the nucleoplasm (Figure 22). A spherical spindle forms which consists of many approximately parallel 20 nm spindle microtubules, only a few of which appear to be in direct association with the centriolar complex (Figures 23, 24, and 25). Figure 24 shows the short centriole

with a central hub measuring 0.12 μm long and 20 nm in diameter. The spindle microtubules can neither be considered as divergent nor as convergent toward a centriole as a focus. Rather the spindle microtubules are arranged roughly parallel to each other.

The spindle axis is not always aligned with the long axis of the hypha; it is a matter of chance to section the cell through the spindle axis. Only one clear cut cross section was obtained and the number of spindle microtubules were estimated at about 5,000, far greater than the corresponding number reported by Krishan and Buck (1965) in their study of L-strain fibroblast mitosis.

Although chromosomes are not apparent in thin sections at interphase and early prophase, by early prometaphase chromosomes have become recognizable by their relatively compact structure and their associated chromosome fibers. The nucleoplasmic region also contains a large number of nucleolar granules, 15-20 nm in diameter. No traces of the pars fibrosa remain visible. Whether or not the complete disappearance of the pars fibrosa and the increase in amount of nucleolar granules are related is subject to speculation.

Unlike other eukaryotic organisms, the nuclear envelope in B. ranarum begins to reform in late prometaphase. Small vesicles and short cisternae fuse to form relatively longer

but still discontinuous cisternae (Figure 26). These small vesicles and short cisternae are presumably old envelope fragments because of their location around the nucleoplasm and their relative freedom from ribosomes on their surfaces. The distinct shape of the vesicles also helps to distinguish them from the elements of the generally tubular ER. However, a participation of ER in the early formation of the nuclear envelope cannot be ruled out on the basis of this observation.

4. Metaphase

By early metaphase increasing indications of ER-nuclear envelope associations are seen and a considerable concentration of ER around the periphery of the nucleus develops (Figure 31). The visualization of two juxtaposed centrioles provides evidence that the replication of the centriole has been accomplished (Figure 28, 29, and 30). Chromosomes have become highly condensed and tended to stay close to the metaphase plate. Each consists of two chromatids connected to each other by diffuse chromatin (Figure 32).

The nucleolar materials increase in amount and aggregate throughout metaphase as indicated by both light and electron microscopy (Figures 26, 27, 33, and insets).

In early metaphase the spindle forms with its axis aligned parallel to the hyphal long axis, but in late metaphase the spindle axis reorients almost 90° so that the

metaphase plate comes to lie parallel or nearly parallel to the hyphal axis. It is believed that the time span for this rotation must be short as out of a dozen metaphase nuclei studied in detail only one was found whose configuration suggested that this rotation was in progress (Figure 33).

By late metaphase there is an alignment of all the chromosomes on an extensive elliptical equatorial plate. The spindle is made up of numerous parallel well-defined spindle microtubules at right angles to the equatorial plane (Figure 34). Study of serial sections reveals that the chromosomes occupy the entire metaphase plate and do not form a "Saturn's ring" as suggested by Robinow (1963). On each side of this plate are two elliptical caps made up of condensed nucleolar materials.

Two pairs of centrioles, the members of each pair oriented 90° to each other, are visible in adjacent serial sections of one metaphase nucleus and are presented as insets in Figure 34. One centriole of each pair (presumably the parental centriole) is appressed to the reformed nuclear envelope, and its tubules are parallel to the spindle axis and the spindle microtubules. One would expect the pairs of centrioles to be located at the intersections of the median axis of the spindle with the periphery of the nucleus, but this is not the case. Instead the centrioles

are found on opposite sides of the elliptical nucleus near its long axis (Figure 34).

Spindle microtubules are numerous and clearly parallel to each other rather than convergent toward centrioles. During metaphase there is difficulty in differentiating chromosome fibers from continuous fibers.

The spindle-shaped chromosomes, measuring about 460 nm long and 230 nm in diameter, are made up of two cone-shaped chromatids connected to each other by a diffuse zone of chromosomal fibrils (Figure 35 and 36). The tip portion of the cone of each chromatid forms a relatively light, diffuse, fibrillar kinetochore with which a single chromosome fiber, consisting of a single microtubule, is associated (Figure 35). In Figure 36 this microtubule appears embedded in the kinetochore rather than attached to its tip. Both the kinetochore and the chromosome are made up of fibrils 3-4 nm in diameter. The chromosome number is estimated to be substantial, much greater than the 60 reported by Robinow (1963). As many as 45 have been counted in a single section of a metaphase nucleus (Figure 34).

5. Anaphase

Accompanied by a rotation of the spindle axis from a position nearly perpendicular to the hyphal axis back to an alignment with the hyphal axis, the daughter chromatids of

each chromosome separate from each other at the onset of anaphase. The separation of the daughter chromatids clearly demonstrates the existence of both chromosome fibers and continuous fibers in the mitotic apparatus. Continuous fibers presumably traverse the entire length of the longitudinal spindle axis, as continuous fibers have been followed as much as $4/5$ of this distance (about $4\text{ }\mu\text{m}$) in a single section. Neither the continuous fibers nor the chromosome fibers have ever been found to terminate on the nuclear envelope; instead they always appear to end about 200 nm away from it.

Serial sectioning of a single nucleus (Figures 40 and 41) reveals that the paired centrioles assume positions in the nearly median axis of the spindle, but the parallel array of spindle microtubules remains the same as in metaphase, not converging on a centriole as focus.

The association between the chromosome fiber and the kinetochore of the daughter chromosome is evident in Figures 37 and 38 and is similar to that described in metaphase.

Measurements of several metaphase nuclei reveal a chromosome-to-pole distance of about $2.8\text{ }\mu\text{m}$; while the chromosome-to-pole distance based on measurement of several early anaphase nuclei is found to be $2.2\text{ }\mu\text{m}$. The pole-to-pole distance in late anaphase is about two and a half times longer than it is in early anaphase ($14.4\text{ }\mu\text{m}$ vs. $5.4\text{ }\mu\text{m}$)

(Figure 42 vs. Figure 39). Although the shortening of chromosome-to-pole distance may account for the initial separation of chromosomes in early anaphase, elongation of the spindle fibers appear to be primarily responsible for the eventual pulling apart of daughter chromosomes.

By late anaphase the chromosomes become indistinguishable from the nucleolar materials that are concentrated in the polar regions of the nucleus. The two nucleolar masses become clearly recognizable in both light and electron micrographs as separate bodies separated by a region of continuous fibers (Figure 42).

The cisternae of the nuclear envelope which broke down in late prophase and started to reform in metaphase are still relatively discontinuous by early anaphase. By late anaphase they have regained their continuity except in the equatorial region where large gaps remain. Appearing in the interzone between the future daughter nuclei are non-membrane-limited transparent patches, membrane-limited vacuoles, and a small number of membrane-bounded osmiophilic bodies similar to those reported by Robbins and Gonatas (1964b) in anaphase Hela cells.

6. Telophase

Because the nuclear envelope begins to reform early in prometaphase, the classic criterion designating the

beginning of telophase is not appropriate in Basidiobolus ranarum mitosis. As a consequence, the beginning of differentiation between the nucleoplasm and nucleoli, first seen as a further condensation of the nucleolar material and the appearance of patches of less dense nucleoplasm, has, somewhat arbitrarily, been taken as the signal of the initiation of telophase (Figures 43 and 44). By this time progressive separation of the two daughter nuclei has resulted in a distance between them of about 25 μ m which continues to widen. Even before the gap in each nuclear envelope is closed, nuclear pores with well developed plugs are evident. Interzonal fibers have mostly disappeared with some residual fibers remaining around each gap of the nucleus. An abundance of cellular organelles such as ribosomes, mitochondria, microbodies, and membrane-bounded osmiophilic bodies appear in the interzone. The nuclei migrate nearly to opposite ends of the long hyphal cell before cytokinesis takes place. The latter occurs by an ingrowth of a septum as observed in light microscopic studies by Robinow (1963). This stage was not studied in this work.

V. DISCUSSION

A. Fine Structure of the Interphase Nucleolus

These studies have provided strong support for Robinow's proposal (1963) that the so-called intranuclear body or central body in Basidiobolus ranarum is the nucleolus. The fine structure of the nucleolus of Basidiobolus ranarum is similar to nucleoli of root tip cells of Vicia faba (Lafontaine and Chouinard, 1963), Triticum vulgare, and Allium cepa (Lord and Lafontaine, 1969), and Ipheion uniflorum (La Cour and Wells, 1967).

Three of the four major nucleolar components described by Hay (1968) are demonstrated in my study: (1) the pars granulosa consisting of 15-20 nm nucleolar granules; (2) the pars fibrosa consisting of 6-8 nm nucleolar fibrils; (3) the proteinaceous matrix of relatively less dense granules and fibrils. Enzymatic digestion experiments have demonstrated that the nucleolar granular and fibrillar areas contain both protein and RNA (Bernhard and Granboulan, 1968). Biochemical studies reveal that the pars fibrosa has higher protein: RNA ratio (7:1) than the pars granulosa (2:1) (Hyde, Sankaranarayan, and Birnstiel, 1965). The arrangement of the pars fibrosa and the pars granulosa in the nucleolus appears to vary in different species. A typical arrangement in Xenopus

(Hay, 1968) and in Chironomus (Gaudecker, 1967) consists of an outer pars granulosa and an inner pars fibrosa. A precise segregation into an outer and an inner zone was not observed in B. ranarum. Instead, the pars fibrosa appears to be interspersed in the pars granulosa (Figure 2) similar to the arrangement observed by Lord and Lafontaine (1969) in Triticum and Allium.

The existence of DNA within the nucleolus has been demonstrated by autoradiography (Hay and Revel, 1963; Granboulan and Granboulan, 1964, 1965; McLeish, 1968), by enzymatic digestion (Chouinard, 1966a; Bernhard and Granboulan, 1968), and by electron microscopy (Lafontaine, 1968; Bernhard and Granboulan, 1968). Employing enzymatic digestion techniques Chouinard (1966a) provides strong evidence that in contrast to the proteinaceous lacunae in the pars granulosa the lacunae confined to the pars fibrosa contain fibrils 6-10 nm in diameter which are DNase digestable. Furthermore, he is able to show by serial sections that these DNA-containing lacunae are continuous with each other. His report gains support from recent work with high resolution autoradiography showing that nucleolar RNA synthesis takes place in the pars fibrosa and then the newly synthesized RNA moves to the pars granulosa (Gaudecker, 1967).

Studying the nucleolus of Ipheion uniflorum, partially dispersed by "Tween 80", La Cour and Wells (1967) suggest the

relationship of the intranucleolar chromatin to the nucleolar organizing chromosome. They believe the nucleolar organizer simply unfolds and loops into the nucleolus in the regions of the pars fibrosa. They showed that the intranucleolar chromatin and the pars fibrosa together extend into loops when treated with Tween 80, the number of loops corresponding to the number of nucleolar chromosomes.

Lacunae confined to the pars fibrosa are clearly shown in Figure 2, but further studies are needed to determine the nature of the fibrillar structure in the lacunae. If La Cour and Wells' conclusion is held for Basidiobolus, it is difficult to visualize the association of chromatin with the pars fibrosa in view of the huge extent of the nucleolus contrasted to the extremely minute size of the chromosomes. Unfortunately the chromosomes are indistinguishable from the rest of the nucleoplasm at the times when the nucleolus is in its mature form. Possibly there are many nucleolar organizing chromosomes associated with the pars fibrosa. It is speculated that these chromosomes are the nucleolar organizers and are completely embedded in the pars fibrosa in forming of the DNA-containing lacunae.

B. The Nucleolus during Mitosis

Prophase nucleoli in B. ranarum behave much like those in grasshopper neuroblasts (Stevens, 1965) and in Vicia faba (Lafontaine and Chouinard, 1963). The pars granulosa and the pars fibrosa disperse into 15-20 nm nucleolar granules and 6-8 nm nucleolar fibrils respectively. Part of the pars fibrosa persists up to late prophase while the pars granulosa has either dispersed or disintegrated by this time. By prometaphase even the persistent part of the pars fibrosa has disappeared; in contrast, the corresponding zone in the nucleoli of cultured Chinese hamster cells persists throughout mitosis (Brinkley, 1965). The great increase of nucleolar materials seen in B. ranarum at prometaphase is presumably a process of resynthesis instead of a regrouping of existing dispersed nucleolar granules and fibrils, because in all species studied to date the synthesis of RNA takes place before the new nucleolus is observed (Hsu, Arrighi, Klevecz, and Brinkley, 1965). In addition this is supported by biochemical studies in Hela cells (Perry, 1967). Prenucleolar materials, as observed on the surface of telophase chromosomes of Vicia faba by Lafontaine and Chouinard (1963) and of grasshopper neuroblasts by Stevens (1965) have not been observed in prometaphase and metaphase

chromosomes of B. ranarum. However, further work by Chouinard (1966b) suggests that the material he earlier called "pre-nucleolar" may, in fact, make no direct contribution to the new nucleolus.

C.. Number of Chromosomes

The number of chromosomes in Basidiobolus ranarum can be estimated by a combination of observations and assumptions. The metaphase plate is an elliptical disk with a long axis of 12 μm (measured from Figure 34) and a short axis of about 8-9 μm (estimated from Figure 34). The area of this disk is then approximately 80 μm^2 . Within an area of about 1 μm^2 (the section is about 0.08 μm thick and the metaphase plate 12 μm long, $12 \mu\text{m} \times 0.08 \mu\text{m} = 1 \mu\text{m}^2$) 45 chromosomes are counted, but each chromosome is large enough to appear in three consecutive sections. In other words, there are at least 45 chromosomes in 3 μm^2 of the elliptical metaphase plate. Thus the number of chromosomes is estimated as 1,200. This number appears surprisingly high but is in agreement with the huge number of spindle microtubules observed in prometaphase in view of the fact that in this species there is only one microtubule associated with each divided kinetochore.

D. Centriole

According to recent reviews on centrioles (Bracker, 1967; Newcomb, 1969) the existence of centrioles has been demonstrated only in organisms with a motile stage in their life cycle. The centrioles found in B. ranarum appear to be the first report of centrioles in an organism lacking a motile stage in its life cycle.

It is presumed that the organization of the centriole in B. ranarum includes 9 sets of microtubular triplets arranged in a cylinder surrounding a central complex which, at least, in part includes a central tubule. However, no cross sections have yet been seen that clearly demonstrate this arrangement. The many profiles of centrioles seen to date are in longitudinal or oblique sections and are consistent with this standard arrangement. However, the centriole in B. ranarum has some unique features when it is compared with other types. The standard picture of the centriolar cycle in Hela cells (Robbins, Jentzsch, and Micali, 1968) consists primarily of the following: (a) two orthogonally-arrayed extranuclear centrioles located at one pole of the interphase nucleus; (b) start of separation of the two orthogonal centrioles during G_1 ; (c) replication of each centriole by "budding" during S before they are very far apart; (d) migration of the centriole pairs by early prophase,

both pairs ultimately resting on a common median axis of the nucleus.

In B. ranarum the two centrioles of the interphase nucleus appear to separate and migrate nearly to opposite poles before they replicate. In Figure 2, only one centriole is found near the interphase nucleus and examination of several serial sections on each side of the section bearing the centriole fails to show the other centriole. In the other set of serial sections through an interphase nucleus, Figure 9 shows a centriole in a small depression in the nuclear envelope at one end of the nucleus, and in an adjacent serial section of the same nucleus (Figure 8) the second centriole is seen about 90° around the nucleus from the first one. A complete set of serial sections of the centriole in Figure 9 were carefully examined (Figure 9, insets a-c) and all of them except a contaminated one (between insets a and b) are presented as insets in Figure 9. Even the contaminated section was meticulously examined and it is apparent that this centriole has not yet replicated.

In early prophase the migrating centrioles have taken positions at opposite poles. In a number of different cells at prophase and metaphase these two centrioles were seen to share a common tangential axis which was parallel but not coincident with the median nuclear axis.

Paired centrioles were not seen until early metaphase; however, no centrioles were found in sections of cells in late prometaphase. To hazard a guess, the replication of centrioles may take place in either late prometaphase or early metaphase. The orthogonal arrays of the parent and daughter centrioles found in metaphase suggest a budding replication mechanism similar to that reported by Dippel (1968). The centrioles were never seen end-to-end as proposed as the replication position in Albugo (Berlin and Bowen, 1964).

E. Rotating Phase

The positions of the two pairs of centrioles in late metaphase provide some clues to the mechanism of the two separate nuclear rotation events observed in mitosis—the rotation taking place between early metaphase and late metaphase, and the reverse rotation between late metaphase and anaphase.

In prometaphase the spindle forms parallel with the hyphal axis and it is roughly oriented between the centrioles near the poles of the spherical nucleus. The tubules of the centrioles are perpendicular to the nuclear envelope and parallel to both the spindle and hyphal long axes. By late metaphase the nucleus has rotated roughly 90° . The

centrioles have not changed their positions in the hypha and each lies near the intersections of the long axis of the now elliptical nucleus and the nuclear envelope. The centrioles have also rotated so that the tubules of each parent centriole are still parallel to the spindle axis, in other words, perpendicular to the hyphal long axis.

Between late metaphase and anaphase the spindle and chromosomes rotate back to a position with the spindle long axis parallel with the hyphal long axis. At the same time the paired centrioles retain their positions relative to the hypha, however, the parent centrioles are seen in their original orientation, i.e., parallel to the hyphal and spindle long axis.

Thus the rotation of the spindle coincide with the orientation of the parent centrioles and it seems possible that one rotation results in the other. However, the orientation of the parent centrioles resulting in the rotation of the spindle is preferred, since the centriole has long been known as an effective organizing center for spindle microtubules (Inoue and Sato, 1967).

F. Nuclear Division

Based on general observations of somatic nuclear divisions in animals and in higher plants, Mazia (1961)

summarizes the essential steps defining mitosis as following: (1) duplication of chromosomes; (2) condensation of chromosomes and breakdown of nucleolus; (3) separation and movement of daughter chromosomes to opposite poles; (4) cytokinesis and reformation of two daughter nuclei. Jenkins (1967) considers the alignment of chromosomes at the equatorial plate of the spindle as an additional, essential step to this definition.

It has become increasingly evident that a wide variety of exceptions to this definition do exist as studies of nuclear division have been extended to a broad spectrum of organisms.

Chromosomes may maintain the condensed form throughout the cell cycle as in the dinoflagellate Gyrodinium cohnii (Kubai and Ris, 1969), or never go through the coiling cycle to form visible chromosomes as in the yeast Saccharomyces (Robinow and Marak, 1966) and in the water mold Blastocladiella (Lessie and Lovett, 1968). Chromosomes observed in the green alga Chlamydomonas apparently lack the compactness that condensed chromosomes normally have (Johnson and Porter, 1968). Nucleolar breakdown does not always accompany condensation of chromosomes. For example, in Psilotum the nucleolus persists to telophase (Allen and Bowen, 1966). Persistent nucleoli are commonly found in animals (Hsu et al., 1964, 1965; Brinkley, 1965; Heneen and

Nichols, 1966) and in plants (Brown and Emery, 1957; Godward and Jordan, 1965). Intranuclear mitoses where the envelope remains intact throughout mitosis are frequently found in fungi such as Albugo candida (Berlin and Bowen, 1964), Blastocladiella (Lessie and Lovett, 1968), Catenaria (Ichida and Fuller, 1968), Saccharomyces spp. (Robinow and Marak, 1966), in the green alga Chlamydomonas (Johnson and Porter, 1968), and in the dinoflagellate Gyrodium (Kubai and Ris, 1969). In many coenocytic organisms and tissues mitosis is not followed by cytokinesis, for example, Albugo candida (Berlin, 1964).

Roth (1964) has managed to group then-known various forms of cell divisions into four categories: (1) anastral mitosis: lack of centrioles and asters; (2) astral mitosis: with centrioles and asters; (3) intranuclear mitosis: basically anastral but nuclear envelope intact; (4) amitosis: direct division of the nucleus without apparent separation of daughter chromosomes. Obviously Roth's classification system is not thorough enough to include all the variations in nuclear division reported since. Rather than coin a specific term for a particular variation of mitosis, Jenkins (1967) refers to all somatic nuclear divisions in eucaryotes as mitosis but recognizes the existence of many variations. He suggests the application of two hyphenated adjectives to describe this variability. The terms "closed" and "open"

are used for an intact or disrupted nuclear envelope respectively, and the terms "centric" and "acentric" for the presence or absence of centrioles respectively. Thus, mitosis in the Blepharisma micronucleus would be "closed-acentric" mitosis; Hela cells "open-centric" mitosis; and, in Albugo "closed-centric" mitosis. This seems useful; however, the way he labels the anaphase nucleus of Physarum flavicomum as "open", simply because there is a gap in the nuclear envelope at each pole, introduces some ambiguity, since Johnson and Porter (1968), using the same terminology, refer to the fenestrated mitotic nucleus in Chlamydomonas as "closed". The term "fenestrated" is recommended here as an adjective to describe the Chlamydomonas-type mitotic nucleus where the envelope remains intact except for the occurrence of several openings larger than nuclear pores. As a result, mitosis in Chlamydomonas would be described as "fenestrated-acentric".

The electron microscopic studies of Basidiobolus ranarum reveal that the nuclear envelope breaks down in early prometaphase and in the course of metaphase the nuclear envelope reforms by the elaboration of envelope remnants and probably ER into a system of more or less discontinuous plate-like cisternae. During anaphase the cisternae gradually fuse as the system gains continuity.

Thus between prometaphase and telophase the nuclear envelope in Basidiobolus ranarum is relatively persistent in contrast to the "open" system. It is recommended that "semi-persistent" is an appropriate term to describe the state of the nuclear envelope in Basidiobolus ranarum. A semi-persistent nuclear envelope during mitosis is also found in Physarum polycephalum. In this species the nuclear envelope breaks down during early prophase to form many cisterna-like envelope fragments that persist through mitosis in a zone around the mitotic apparatus and participate in the formation of a new nuclear envelope in telophase (Guttes, Guttes, and Ellis, 1968). Similarly in rat thymic lymphocytes (Murray, Murray, and Pizzo, 1965), in giant amoebae (Daniels and Roth, 1964), and in Amoeba proteus (Roth, Obetz, and Daniels, 1960), large pieces of the nuclear envelope persist through mitosis and possibly take part in the formation of a new nuclear envelope during telophase. The term semi-persistent seems also appropriate to describe the state of the nuclear envelope in these organisms.

The terms centric and acentric appear to become too restrictive in their original definition since several kinds of organizing centers for spindle microtubules appear to exist in fungi. For example, the centrosomal masses in Coprinus (Lu, 1967), Armillaria (Motta, 1967) and Polystictus

(Burnett, 1968), the centriolar plaques in yeast (Robinow and Marak, 1966) and Pustularia (Schrantz, 1967), more or less conventional tubular centrioles in Albugo (Berlin and Bowen, 1964), Catenaria (Ichida and Fuller, 1968), and here in Basidiobolus all appear to have real differences. It is proposed here that the terms "centric" and "acentric" apply not just to mitoses in cells with or without conventional centrioles respectively but instead be modified to indicate the presence or absence of any organizing center for spindle microtubules. As a result mitosis in Basidiobolus ranarum is properly described as semi-persistent-centric and in yeast closed-centric.

A comparison of the light microscopic observations of B. ranarum by Robinow (1963) and the electron microscopic observations reported here is summarized in Table 2.

Table 2. Differences between LM and EM observations of Basidiobolus ranarum

Feature	LM studies (Robinow, 1963)	EM studies (this work)
Mitosis	open-acentric	semi-persistent-centric
Centrioles	absent	present
Organizing centers of spindle microtubules	nucleolus	centrioles
Nucleolus	persistent	not persistent
Arrangement of metaphase chromosomes	"Saturn's ring"	disk-like
Rotating phase	absent	present

Table 2. (Continued)

Feature	LM studies (Robinow, 1963)	EM studies (this work)
"End plates"	parts of the original nucleolus	reformed nucleoli
No. of chromosomes	60	much more than 60
Kinetochores	not observed	present
No. of spindle micro-tubules per kinetochore	not observed	always one

VI. SUMMARY

1. The culture of Basidiobolus ranarum Eidam mycelia on agar-coated mica slides coupled with an open-face embedding technique permitted an ultrastructural study of nuclear division in somatic hyphal cells of this fungus.

2. Despite previous reports to the contrary, perinuclear centrioles are present in somatic hyphal cells and the division and subsequent behavior of these centrioles is correlated with the several stages of mitosis.

3. During interphase the two tiny centrioles have already separated and migrated apart; the first one is seen at one pole of the spindle-shaped interphase nucleus, while the second one is about 90° around the nucleus from the first one.

4. By early prophase these two centrioles are about 150° apart and neither is at a pole of the elongate nucleus. Some extra-nuclear microtubules were seen radiating from each centriole. The nucleolus gradually disperses into scattered nucleolar granules and fibrils until only a small part of the nucleolus remains intact. As prophase progresses, microtubules appear around each pole of the nucleus but they do not converge on the centrioles.

5. By prometaphase the complete disorganization of the nuclear envelope coincides with the disappearance of the

pars fibrosa component of the nucleolus and an increase in amount of the nucleolar materials.

Chromosomes and spindle microtubules become recognizable. From late prometaphase to the time of nuclear envelope reorganization in telophase envelope fragments and possibly elements of the ER aggregate in a roughly concentric array around the nuclear area.

6. By early metaphase the centrioles have replicated. After the mitotic apparatus accomplishes a 90° rotation to put the spindle axis perpendicular to the hyphal long axis, the metaphase chromosomes orderly align on an equatorial plate. Throughout this nuclear rotation the paired centrioles retain their positions relative to the hyphal axis. The parent centrioles, however, appear to rotate 90° at the same time the nucleus rotates and are always parallel to the spindle axis.
7. Anaphase is immediately preceded by a reverse rotation of the nucleus back to its original orientation in the hypha. The centrioles, again keep their positions relative to the hypha and the nucleus, but rotate to maintain the axis of the parent centrioles parallel with the spindle axis.

The sister chromatids separate from each other in anaphase.

8. By the beginning of telophase the pole-to-pole distance has reached 25 μm and continues to expand. The disappearance of the chromosomes from view and the differentiation of nucleoplasm and nucleolus in each separate nucleus mark the beginning of telophase. In telophase the nuclear plugs make their first appearance in well-defined nuclear pores of the nuclear envelope. The latter almost closes now except an occasional gap where some residual continuous fibers remain visible. Cytokinesis is accomplished by an ingrowth of the cell wall, the septum, after the two daughter nuclei have widely separated.

VII. LITERATURE CITED

- Aist, J. R. 1969. The mitotic apparatus in fungi, Ceratocystis fagacearum and Fusarium oxysporum. Journal of Cell Biology 40: 120-135.
- Aist, J. R. and C. L. Wilson. 1967. Nuclear behavior in the vegetative hyphae of Ceratocystis fagacearum. American Journal of Botany 54: 99-104.
- Aldrich, H. C. 1969. The ultrastructure of mitosis in myxamoebae and plasmodia of Physarum flavicomum. American Journal of Botany 56: 290-299.
- Allen, R. D. 1969. The morphogenesis of basal bodies and accessory structures of the cortex of the ciliated protozoan Tetrahymena pyriformis. Journal of Cell Biology 40: 716-733.
- Allen, R. D. and C. C. Bowen. 1966. Fine structure of Psilotum nudum cells during division. Caryologia 19: 299-342.
- Amano, S. 1957. The structure of the centrioles and spindle body as observed under the electron and phase contrast microscopies. A new extension-fiber theory concerning mitotic mechanism in animal cells. Cytologia 22: 193-212.
- Anderson, W. A. and R. A. Ellis. 1965. Ultrastructure of Trypanosoma lewisi: flagellum, microtubules, and the kinetoplast. Journal of Protozoology 12: 483-499.
- Bakerspiegel, A. 1957. The structure and mode of division of the nuclei in the yeast cells and mycelium of Blastomyces dermatitidis. Canadian Journal of Microbiology 3: 923-933.
- Bakerspiegel, A. 1958. The structure and mode of division of the nuclei in the vegetative spores and hyphae of Endogone sphagnophila ATK. American Journal of Botany 45: 404-410.
- Bakerspiegel, A. 1959a. The structure and manner of division of the nuclei in the vegetative mycelium of the basidiomycete Schizophyllum commune. Canadian Journal of Botany 37: 835-842.

- Bakerspiegel, A. 1959b. The structure and manner of division of the nuclei in the vegetative mycelium of Neurospora crassa. American Journal of Botany 46: 180-190.
- Bakerspiegel, A. 1959c. The structure and manner of division of the nuclei in the vegetative mycelium of Gelasinospora tetrasperma. Canadian Journal of Microbiology 5: 125-130.
- Bakerspiegel, A. 1960a. Nuclear structure and division in vegetative mycelium of the Saprolegniaceae. American Journal of Botany 47: 94-100.
- Bakerspiegel, A. 1960b. Nuclear structure and division in the fungi imperfecti: 11. Scopulariopsis brevicaulis. Cytologia 25: 344-351.
- Bakerspiegel, A. 1961. Vegetative nuclear division in the ascomycete Ophiostoma fimbriata. Cytologia 26: 42-49.
- Bakerspiegel, A. 1964. Some observations on the cytology of Candida albicans. Journal of Bacteriology 87: 228-230.
- Barnicot, N. A. and H. E. Huxley. 1965. Electron microscope observations on mitotic chromosomes. Quarterly Journal of Microscopical Science 106: 197-214.
- Berkaloff, C. 1963. Les cellules méristématiques d'Himantalia lorea (L.) S. F. Gray. Étude au microscope électronique. Journal de microscopie 2: 213-228.
- Berlin, J. D. 1964. Flagella initiation and centrioles in Albugo. Unpublished Ph.D. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
- Berlin, J. D. and C. C. Bowen. 1964. Centrioles in the fungus Albugo candida. American Journal of Botany 51: 650-652.
- Bernhard, W. and E. de Harven. 1960. L'ultrastructure du centriole et d'autres éléments de l'appareil achromatique. International Conference on Electron Microscopy, 4th, Berlin, 1958, Proceedings 2: 217-227.
- Bernhard, W. and N. Granboulan. 1968. Electron microscopy of the nucleolus in vertebrate cells. In Dalton, A. J. and F. Haguénau, eds. Ultrastructure in biological systems. Vol. 3. Pp. 81-137. New York, N. Y., Academic Press, Inc.

- Bessis, M. and J. Breton-Gorius. 1957. Le centriole des cellules du sang étude à l'état vivant et au microscope électronique. Bulletin de Microscopie Appliquée 7: 54-56.
- Bloom, W. 1960. Preparation of a selected cell for electron microscopy. Journal of Cell Biology 7: 191-193.
- Borysko, E. and P. Sapranaukas. 1954. A new technique for comparative phase-contrast and electron microscope studies of cells grown in tissue culture with an evaluation of the technique by means of time-lapse cinemicrographs. Johns Hopkins Hospital Bulletin 95: 68-73.
- Bracker, C. E. 1967. Ultrastructure of fungi. Annual Review of Phytophthology 5: 343-374.
- Brinkley, B. R. 1965. The fine structure of the nucleolus in mitotic divisions of Chinese hamster cells in vitro. Journal of Cell Biology 27: 411-422.
- Brinkley, B. R. and E. Stubblefield. 1966. The fine structure of the kinetochore of a mammalian cell in vitro. Chromosoma 19: 28-43.
- Brinkley, B. R., P. Murphy, and L. C. Richardson. 1967. Procedure for embedding in situ selected cells cultured in vitro. Journal of Cell Biology 35: 279-283.
- Brinkley, B. R., E. Stubblefield, and T. C. Hsu. 1967. The effects of colcemid inhibition and reversal on the fine structure of the mitotic apparatus of Chinese hamster cells in vitro. Journal of Ultrastructure Research 19: 1-18.
- Brown, W. V. and E. M. Bertke. 1969. Textbook of Cytology. Saint Louis, Missouri, The C. V. Mosby Co.
- Brown, W. V. and W. H. P. Emery. 1957. Persistent nucleoli and grass systematics. American Journal of Botany 44: 585-590.
- Brushaber, J. A., C. L. Wilson, and J. R. Aist. 1967. Asexual nuclear behavior of some plant-pathogenic fungi. Phytopathology 57: 43-45.
- Burnett, J. H. 1968. Fundamentals of Mycology. London, Britain, Edward Arnold Ltd.

- Chouinard, L. A. 1966a. Localization of intranucleolar DNA in root meristematic cells of Allium cepa. *Journal of Cell Biology* 31: 139A.
- Chouinard, L. A. 1966b. Nucleolar architecture in root meristematic cells of Allium cepa. *National Cancer Institute Monograph* 23: 125-143.
- Chouinard, L. A. and C. P. Leblond. 1967. Sites of protein synthesis in nucleoli of root meristematic cells of Allium cepa as shown by radioautography with (^3H)-arginine. *Journal of Cell Science* 2: 473-480.
- Daniels, E. W. and L. E. Roth. 1964. Electron microscopy of mitosis in radiosensitive giant amoeba. *Journal of Cell Biology* 20: 75-84.
- de Harven, E. 1968. The centriole and the mitotic spindle. In Dalton, A. J. and F. Haguénau, eds. *Ultrastructure in biological systems*. Vol. 3. Pp. 197-224. New York, N.Y., Academic Press, Inc.
- de Harven, E. and W. Bernhard. 1956. Étude au microscope électronique de l'ultrastructure du centriole chez les vertébrés. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* 45: 378-398.
- de Thé, G. 1964. Cytoplasmic microtubules in different animal cells. *Journal of Cell Biology* 23: 265-275.
- Dippel, R. V. 1968. The development of basal bodies in Paramecium. *National Academy of Sciences Proceedings* 61: 461-468.
- Dirksen, E. R. and T. T. Crocker. 1965. Centriole replication in differentiating cells of mammalian respiratory epithelium. An electron microscope study. *Journal of Microscopie* 5: 629-644.
- Dowding, E. S. and J. Weiher. 1960. Mitosis in Neurospora. *Nature* 188: 338-339.
- Dowding, E. S. and J. Weiher. 1962. Mitosis in Neurospora and Gelasinospora. 1. *Genetica* 32: 339-351.
- Duncan, E. J. and J. A. MacDonald. 1965. Nuclear phenomena in Marasmius androsaceus and Marasmius rotula. *Royal Society of Edinburgh Transactions* 66: 129-141.

- Eidam, E. 1887. Basidiobolus, eine neue Gattung der Entomorphothoraceen. Beitrage zur Biologie der Pflanzen 4: 181-251.
- Gall, J. G. 1961. Centriole replication-A study of spermatogenesis in snail Viviparus. Journal of Biophysical and Biochemical Cytology 10: 163-193.
- Gaudecker, B. V. 1967. RNA synthesis in the nucleolus of Chironomus thummi, as studied by high resolution autoradiography. Zeitschrift für Zellforschung 82: 536-557.
- Gay, H. 1955. Serial sections of smears for electron microscopy. Stain Technology 30: 239-242.
- Geuskens, M. and W. Bernhard. 1966. Cytochimie ultra-structurale du nucléole. III. Action de l'actinomycine D sur le métabolisme du RNA nucléolaire. Experimental Cell Research 44: 579-598.
- Girbardt, M. 1962. Licht und Elektronenmikroskopische Untersuchungen an Polystictus versicolor. VIII. Faerberische analyse der vegetativen Kernteilung. Planta 58: 1-21.
- Godward, M. B. and E. G. Jordan. 1965. Electron microscopy of the nucleolus of Spirogyra britannica and Spirogyra ellipsospora. Royal Microscopical Society Journal 84: 347-360.
- Granboulan, N. and P. Granboulan. 1964. Cytochimie ultra-structurale du nucléole. I. Mise en évidence de chromatine à l'intérieur du nucléole. Experimental Cell Research 34: 71-87.
- Granboulan, N. and P. Granboulan. 1965. Cytochimie ultra-structurale du nucléole. II. Étude des sites de synthèse du RNA dans le nucléole et le noyau. Experimental Cell Research 38: 604-619.
- Guttes, S., E. Guttes, and R. A. Ellis. 1968. Electron microscope study of mitosis in Physarum polycephalum. Journal of Ultrastructure Research 22: 508-529.
- Hay, E. D. 1968. Structure and function of the nucleolus in developing cells. In Dalton, A. J. and F. Haguenau, eds. Ultrastructure in biological system. Vol. 3. Pp. 2-70. New York, N.Y., Academic Press, Inc.

- Hay, E. D. and J. P. Revel. 1963. The fine structure of the DNP component of the nucleus. An electron microscopic study utilizing autoradiography to localize DNA synthesis. *Journal of Cell Biology* 16: 29-51.
- Hartman, G. C. 1964. Nuclear division in Alternaria tenuis. *American Journal of Botany* 51: 209-212.
- Heale, J. B., A. Gafoor, and K. C. Rajasinghan. 1968. Nuclear division in conidia and hyphae of Verticillium albo-atrum. *Canadian Journal of Genetics and Cytology* 10: 321-340.
- Heneen, W. K. and Nichols, W. W. 1966. Persistence of nucleoli in short term and long term cell cultures and in direct bone marrow preparations in mammalian materials. *Journal of Cell Biology* 31: 543-561.
- Heyner, S. 1963. In situ embedding of cultured cells or tissue, grown on glass, in epoxy resins for electron microscopy. *Stain Technology* 38: 335-336.
- Howatson, A. F. and J. D. Almeida. 1958. A method for the study of cultured cells by thin sectioning and electron microscopy. *Journal of Biophysical and Biochemical Cytology* 4: 115-117.
- Hsu, T. C., F. E. Arrighi, R. R. Klevecz, and B. R. Brinkley. 1965. The nucleoli in mitotic divisions of mammalian cells in vitro. *Journal of Cell Biology* 26: 539-553.
- Hsu, T. C., B. R. Brinkley, and F. E. Arrighi. 1967. The structure and behavior of the nucleolus organizers in mammalian cells. *Chromosoma* 23: 137-153.
- Hsu, T. C., R. M. Humphrey, and C. E. Somers. 1964. Persistent nucleoli in animal cells following treatments with fluorodeoxyuridine and thymidine. *Experimental Cell Research* 33: 74-77.
- Hyde, B. B., K. Sankaranarayanan, and M. L. Birnstiel. 1965. Observations on fine structure in pea nucleoli in situ and isolated. *Journal of Ultrastructure Research* 12: 652-667.
- Ichida, A. A. and M. S. Fuller. 1968. Ultrastructure of mitosis in the aquatic fungus Catenaria anguillulae. *Mycologia* 60: 141-155.

- Inoue, S. and H. Sato. 1967. Cell motility by labile association of molecules. *Journal of General Physiology* 50: 259-292.
- Jenkins, R. A. 1967. Fine structure of division in ciliate protozoa. 1. Micronuclear mitosis in Blepharisma. *Journal of Cell Biology* 34: 463-481.
- Jensen, J. and A. Bajer. 1969. Effects of dehydration on the microtubules of the mitotic spindle studies in vitro and with the electron microscope. *Journal of Ultrastructure Research* 26: 367-386.
- Johnson, U. G. and K. R. Porter. 1968. Fine structure of cell division in Chlamydomonas reinhardi. *Journal of Cell Biology* 38: 403-425.
- Knox-Davis, P. S. 1968. Nuclear division in the developing pycnospores of Macrophomina phaseoli. *American Journal of Botany* 53: 220-224.
- Krishan, A. and R. C. Buck. 1965. Structure of the mitotic spindle in L-strain fibroblasts. *Journal of Cell Biology* 24: 433-444.
- Kubai, D. F. and H. Ris. 1969. Division in the dinoflagellate Gyrodinium cohnii. *Journal of Cell Biology* 40: 508-528.
- Laane, M. M. 1967. The nuclear division in Penicillium expansum. *Canadian Journal of Genetics and Cytology* 9: 342-351.
- LaCour, L. F. and B. Wells. 1967. The loops and ultrastructure of the nucleolus of Ipheion uniflorum. *Zeitschrift für Zellforschung* 82: 25-45.
- Lafontaine, J. G. 1968. Structural components of the nucleus in mitotic plant cells. In Dalton, A. J. and F. Haguenau, eds. *Ultrastructure in biological system*. Vol. 3. Pp. 151-196.
- Lafontaine, J. G. and L. A. Chouinard. 1963. A correlated light and electron microscope study of the nucleolar material during mitosis in Vicia faba. *Journal of Cell Biology* 17: 167-201.

- Latta, H. 1959. A cellular reaction to antibody in tissue culture studied with electron microscopy. *Journal of Biophysical and Biochemical Cytology* 5: 405-410.
- Ledbetter, M. and K. R. Porter. 1963. A microtubule in plant cell fine structure. *Journal of Cell Biology* 19: 239-250.
- Lessie, E. and J. S. Lovett. 1968. Ultrastructure changes during sporangium formation and zoospore differentiation in Blastocladiella emersonii. *American Journal of Botany* 55: 220-236.
- Lord, A. and J. G. Lafontaine. 1969. The organization of the nucleolus in meristematic plant cells. A cytochemical study. *Journal of Cell Biology* 40: 633-647.
- Lu, B. C. 1967. Meiosis in Coprinus lagopus: A comparative study with light and electron microscopy. *Journal of Cell Science* 2: 529-536.
- Lwoff, A. 1950. Problems of morphogenesis in ciliates. New York, N.Y., Wiley.
- Mazia, D. 1961. Mitosis and the physiology of cell division. In Brachet, J. and A. E. Murphy, eds. *The cell*. Vol. 3. Pp. 77-394. New York, N.Y., Academic Press, Inc.
- McLeish, J. 1968. Chemical and autoradiographic studies of intranucleolar DNA in Vicia faba. *Experimental Cell Research* 51: 157-166.
- Mizukami, I. and J. Gall. 1966. Centriole replication. 11. Sperm formation in the fern, Marsilea and the cycad, Zamia. *Journal of Cell Biology* 29: 97-111.
- Moor, H. 1966. Ultrastrukturen in Zellkern der Backerhefe. *Journal of Cell Biology* 29: 153-155.
- Moore, R. T. 1964. Fine structure of mycota. 12. Karyochinesis-somatic nuclear division — in Cordyceps militaris. *Zeitschrift für Zellforschung* 63: 921-937.
- Motta, J. J. 1967. A note on the mitotic apparatus in the rhizomorph meristem of Armillaria mellea. *Mycologia* 59: 370-375.

- Murray, R. G., A. S. Murray, and A. Pizzo. 1965. The fine Structure of mitosis in rat thymic lymphocytes. *Journal of Cell Biology* 26: 601-619.
- Namboodiri, A. N. and R. J. Lowry. 1967. Vegetative nuclear division in Neurospora. *American Journal of Botany* 54: 735-748.
- Newcomb, E. H. 1969. Plant microtubules. *Annual Review of Plant Physiology* 20: 253-288.
- Newcomb, E. H. and H. T. Bonnett. 1965. Cytoplasmic microtubule and wall microfibril orientation in root hairs of radish. *Journal of Cell Biology* 27: 575-589.
- Olive, E. W. 1907. Cell and nuclear division in Basidiobolus. *Annals of Mycology* 5: 404-418.
- Perry, R. P. 1967. The nucleolus and the synthesis of ribosomes. In Davidson, J. N. and W. E. Cohn, eds. *Progress in nucleic acid research and molecular biology*. Vol. 6. pp. 219-257.
- Persijn, J. P. and J. P. Scherft. 1965. Sheet mica— A non-adherent carrier for surface culture of cells to be embedded in Epon. *Stain Technology* 40: 89.
- Phillips, D. M. 1967. Giant centriole formation in Sciara. *Journal of Cell Biology* 33: 73-92.
- Raciborski, M. 1896. Uber Einfluss aussern Bekingungen auf die Wachstumsweise des Basidiobolus ranarum. *Flora* 82: 107-132.
- Renaud, F. L. and H. Swift. 1964. The development of basal bodies and flagella in Allomyces arbuscula. *Journal of Cell Biology* 23: 339-354.
- Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* 17: 208-212.
- Robbins, E. and N. K. Gonatas. 1964a. In vitro selection of the mitotic cell for subsequent electron microscopy. *Journal of Cell Biology* 20: 356-359.
- Robbins, E. and N. K. Gonatas. 1964b. The ultrastructure of a mammalian cell during the mitotic cycle. *Journal of Cell Biology* 21: 429-463.

- Robbins, E., G. Jentzsch, and A. Micali. 1968. The centriole cycle in synchronized Hela cells. *Journal of Cell Biology* 36: 329-339.
- Robinow, C. F. 1957. The structure and behavior of the nuclei in spores and growing hyphae of Mucorales. 1. *Canadian Journal of Microbiology* 3: 771-789.
- Robinow, C. F. 1961. Mitosis in the yeast Lipomyces lipofer. *Journal of Biophysical and Biochemical Cytology* 9: 879-892.
- Robinow, C. F. 1962. Some observations on the mode of division of somatic nuclei of Mucor and Allomyces. *Archiv für Mikrobiologie* 42: 369-377.
- Robinow, C. F. 1963. Observations on cell growth, mitosis, and division in the fungus Basidiobolus ranarum. *Journal of Cell Biology* 17: 123-152.
- Robinow, C. F. and A. Bakerspiegel. 1965. Somatic nuclei and forms of mitosis in fungi. In Ainsworth, G. C. and A. S. Sussaman, eds. *The fungi*. Vol. 1. Pp. 119-142. New York, N.Y., Academic Press, Inc.
- Robinow, C. F. and J. A. Marak. 1966. A fiber apparatus in the nucleus of the yeast cell. *Journal of Cell Biology* 29: 129-151.
- Roth, L. E. 1964. Motile systems with continuous filaments. In Allen, R. D. and N. Kamiya, eds. *Primitive motile systems in cell biology*. Pp. 527-548. New York, N.Y., Academic Press, Inc.
- Roth, L. E., S. W. Obetz, and E. W. Daniels. 1960. Electron microscopic studies of mitosis in Ameebae 1. Amoeba proteus. *Journal of Biophysical and Biochemical Cytology* 8: 207-220.
- Saskena, H. K. 1961. Nuclear structure and division in the mycelium and basidiospores of Ceratobasidium praticolum. *Canadian Journal of Botany*: 749-756.
- Schrantz, J. P. 1967. Presence d'un aster au cours des mitosis de l'asque et de la formation des ascospores chez l'Ascomycete Pustularia cupularis (L.) Fuck. *Academie des Sciences, Paris, Series D* 264: 1274-1277.
- Slifkin, M. K. 1967. Nuclear division in Saprolegnia as revealed by colchicine. *Mycologia* 59: 431-445.

- Smith-Sonneborn, J. and W. Plaut. 1967. Evidence for the presence of DNA in the pellicle of Paramecium. Journal of Cell Science 2: 225-234.
- Somers, C. E., R. P. Wagner, and T. C. Hsu. 1960. Mitosis in vegetative nuclei of Neurospora crassa. Genetics 45: 801-810.
- Sorokin, S. 1962. Centrioles and the formation of rudimentary cilia by fibroblasts and smooth muscle cells. Journal of Cell Biology 15: 363-377.
- Stempak, J. G. and R. T. Ward. 1964. An improved staining method for electron microscopy. Journal of Cell Biology 22: 697-701.
- Stevens, B. J. 1965. The fine structure of the nucleolus during mitosis in the grasshopper neuroblast cell. Journal of Cell Biology 24: 349-368.
- Stobb, R., R. L. Amy, R. K. Wertz, B. Fauconnier, and M. Bessis. 1966. An electron microscope study of vitally stained single cells irradiated with ruby laser microbeam. Journal of Cell Biology 31: 11-29.
- Stubblefield, E. and B. R. Brinkley. 1966. Cilia formation in Chinese hamster fibroblasts in vitro as a response to colcemid treatment. Journal of Cell Biology 30: 645-652.
- Stubblefield, E. and B. R. Brinkley. 1967. Architecture and function of the mammalian centriole. In Warren, K. B., ed. Formation and fate of cell organelles. Pp. 175-218. New York, N.Y., Academic Press, Inc.
- Sutton, J. S. 1965. In situ embedding in epoxy resin of tissue cultured in Leighton tubes, selection of single cells for electron microscopy. Stain Technology 40: 151-155.
- Thaxter, R. 1888. The Entomorphthoreae of the United States. Memoirs of Boston Society of Natural Sciences 4: 133-201.
- Thyagarajan, T. R. and H. B. Naylor. 1962. Cytology of Rhodotorula glutinis. Journal of Bacteriology 83: 127-136.

- Turian, G. and E. C. Cantino. 1960. A study of mitosis in the mold Blastocladiella with a ribonuclease-aceto-orcein staining technique. *Cytologia* 25: 101-107.
- Turner, F. R. 1968. An ultrastructural study of plant spermatogenesis. Spermatogenesis in Nitella. *Journal of Cell Biology* 37: 370-393.
- Ward, E. W. B. and K. W. Ciurysek. 1962. Somatic mitosis in Neurospora crassa. *American Journal of Botany* 49: 393-399.
- Weijer, J., A. Koopmans, and D. L. Weijer. 1963. Karyokinesis in vivo of the migrating somatic nucleus of Neurospora and Gelasinospora species. *Transactions of New York Academy of Sciences* 25: 846-854.
- Weijer, J., A. Koopmans, and D. L. Weijer. 1965. Karyokinesis of somatic nuclei of Neurospora crassa: 111. The juvenile and maturation cycles (Feulgen and crystal violet staining). *Canadian Journal of Genetics and Cytology* 7: 140-163.
- Weijer, J., and B. R. MacDonald. 1965. Karyokinesis of somatic nuclei of Neurospora crassa. IV. The occurrence of the juvenile ring phase during division IV of ascosporeogenesis. *Canadian Journal of Genetics and Cytology* 7: 519-522.
- Weijer, J. and S. H. Weisberg. 1966. Karyogenesis of the somatic nucleus of Aspergillus nidulans. 1. The juvenile chromosome cycle (Feulgen staining). *Canadian Journal of Genetics and Cytology* 8: 361-374.
- Widra, A. 1959. Studies on the nucleus of Candida spp. *Mycopathologia et Mycologia Applicata* 11: 177-154.
- Yamada, E. 1958. Some observations on the fine structure of centriole in the mitotic cell. *Journal of Krume Medicine* 5: 36-74.
- Yoneda, Y. 1963. Karyological studies on yeast. *Cytologia* 28: 131-145.

VIII. ACKNOWLEDGEMENTS

The author is greatly indebted to his major adviser Dr. C. C. Bowen for his helpful criticism throughout this work. Thanks are also extended to Dr. H. T. Horner, Jr. for his consistent advice and suggestion. The author is also grateful to Mr. J. Braselton for his help to operate the carbon evaporator.

IX. APPENDIX

Explanation of FiguresKey to abbreviations used in figures

CW, cell wall	NP, nuclear pore
C, centriole	NuG, nucleolar granule
Ch, chromosome	NuF, nucleolar fibril
ChF, chromosome fiber	Nu, nucleolus
ChrF, chromosomal fibril	Nm, nucleoplasm
CF, continuous fiber	N, nucleus
CyI, cytoplasmic invagination	Fib, pars fibrosa
ER, endoplasmic reticulum	Gr, pars granulosa
FB, filament body	PM, proteinaceous matrix
K, kinetochore	SMT, spindle microtubule
MOB, membrane-bounded osmio- philic body	V, vesicles
MP, metaphase plate	
Mb, microbody	
Mt, microtubule	
M, mitochondrion	
MVB, multiple vesicular body	
NE, nuclear envelope	
NEF, nuclear envelope fragment	
NuP, nuclear plug	

Figures 1a-1L. Phase contrast micrographs of mitosis in living hyphae of Basidiobolus ranarum. Figures 1c-1f and Figures 1g-1i represent time lapse sequence of the same cells. Figure 1a. Interphase nucleus consists of a central dark body, the nucleolus (Nu), surrounded by a peripheral bright zone, the nucleoplasm (Nm). Several nucleolar "vacuoles" are visible in the nucleolus. Figure 1b. Interphase nucleus without nucleolar vacuoles in its nucleolus. Figures 1c and 1d. At prometaphase the nucleus rounds up and appears diffuse. No distinction can be made between the nucleolus and the nucleoplasm. Figures 1e and 1f. An early metaphase nucleus showing two elliptical dense bodies on either side of a central bright zone (see arrows), the spindle. Figures 1g and 1h. The metaphase plate (MP) begins to appear in the middle of the spindle. The two dense bodies tend to round up. Figure 1i. At the onset of anaphase the metaphase plate is seen to separate into two discs (see arrows) which move apart toward opposite poles. The two dense bodies have rounded up forming two hemisphere-like caps. Separation of daughter chromosomes appear to result largely from elongation of the interzonal fibers and the separation of daughter nuclei. Figure 1j. The interzonal spindle fibers appear to expand pushing the two dense bodies further apart at late anaphase. Figures 1k and 1L. Many cellular organelles move into the interzone at telophase as the nuclei (N) move apart. The dense bodies resemble nucleoli in the separated nuclei

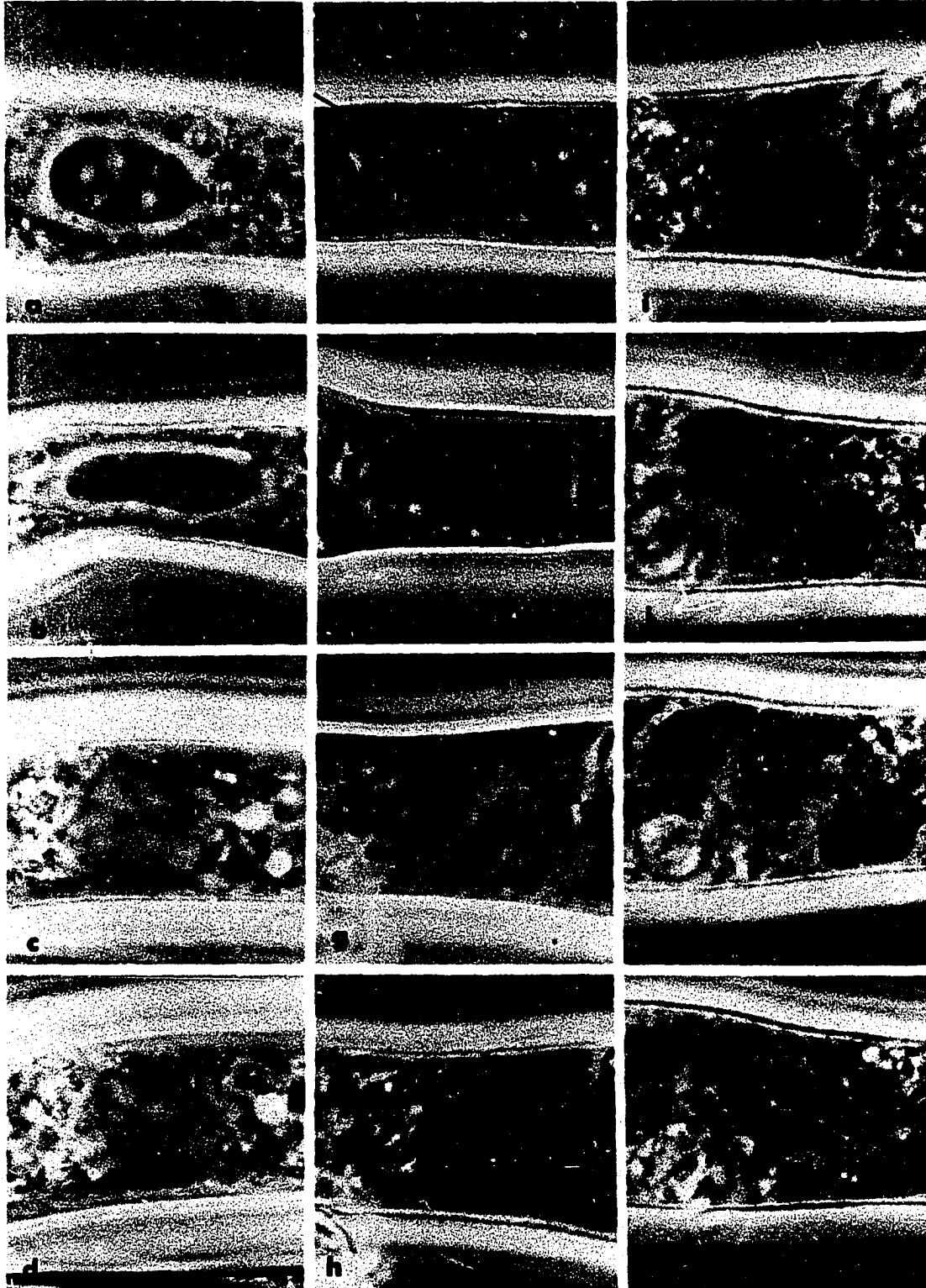


Figure 2. An interphase nucleus, bounded by nuclear envelope (NE) with many dense nuclear plugs (NuP), showing a huge nucleolus (Nu) in the nucleoplasm (Nm). The nucleolus is made up of the pars granulosa (Gr) and the pars fibrosa (Fib). Light patches distributed in the pars fibrosa are the lacunae in which DNA-like materials are present. Within the nucleoplasm a large cytoplasmic invagination (CyI) is visible. A small centriole (C) at one end of the nucleus is magnified in the inset.

Figure 3. An interphase nucleus with two nucleoli (Nu) in which several proteinaceous matrices (PM) are shown. Inset: light micrograph of the same nucleus prior to sectioning

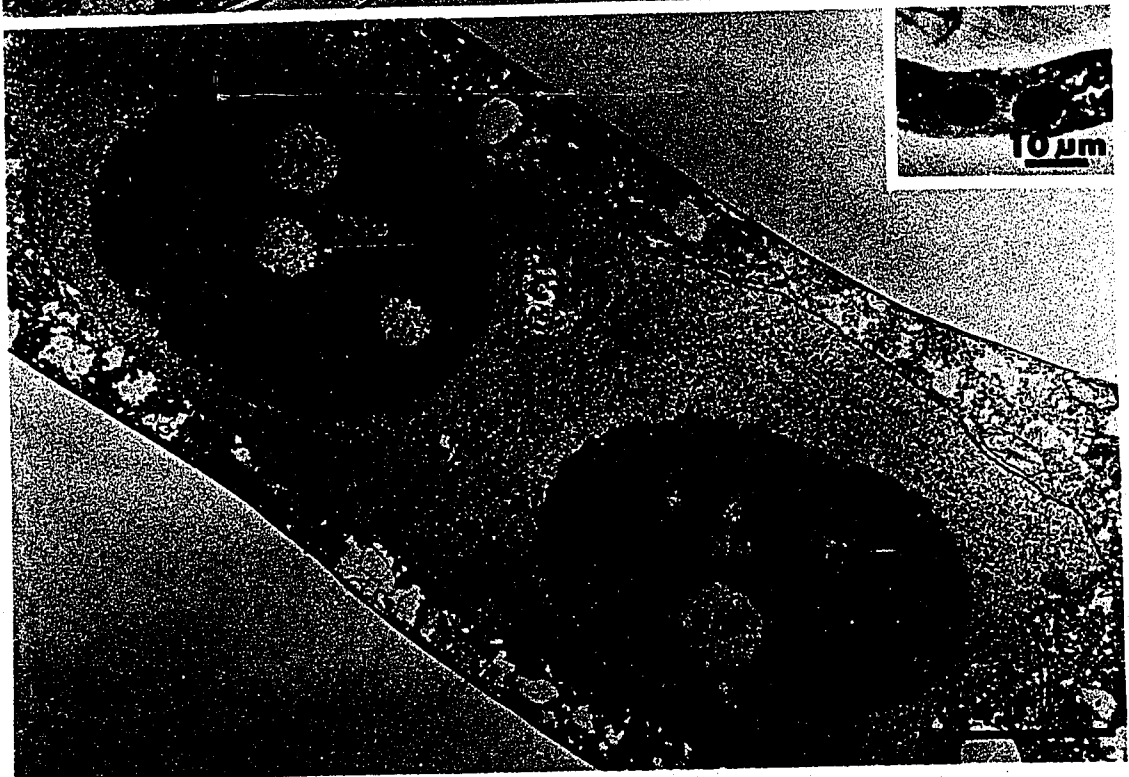
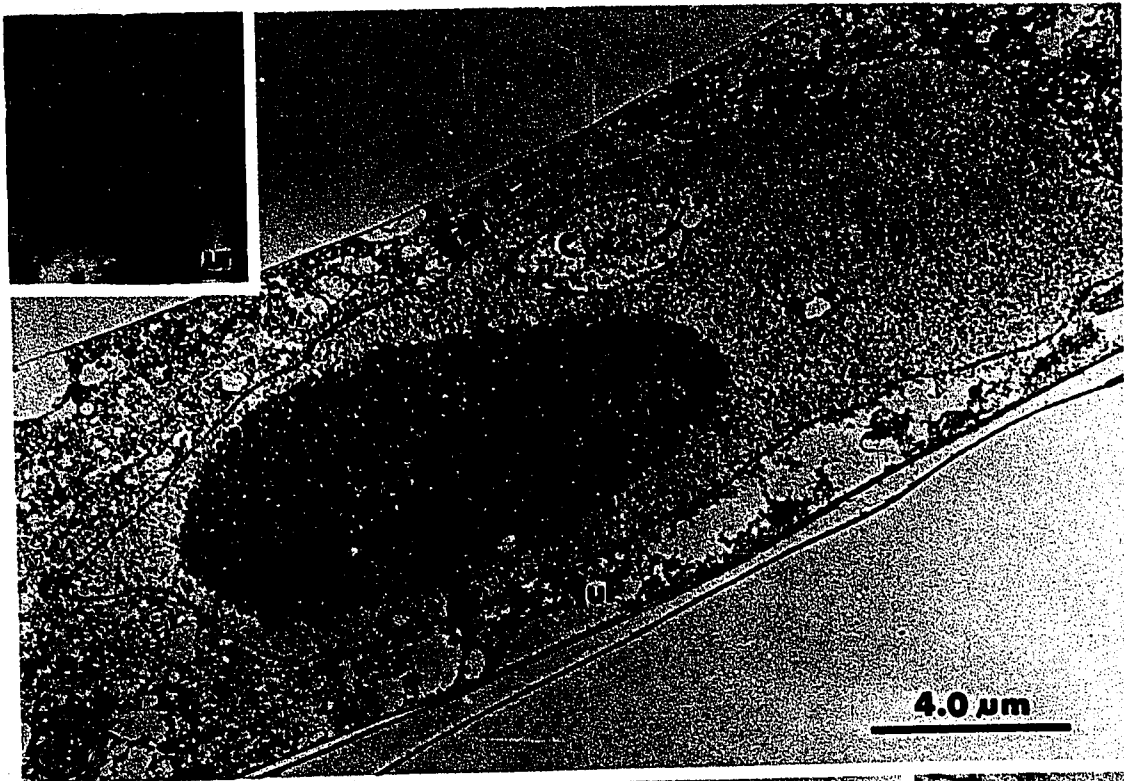


Figure 4. Interphase nucleus with nuclear pores in the nuclear envelope (NE) and nuclear plugs (NuP) in both tangential and cross sections. The large arrow points to a nucleoplasmic fibril and small arrows to aggregates of nucleoplasmic granules

Figure 5. Interphase cell with cytoplasmic microtubules (Mt), about 7 μ m from the cell wall (CW), tubular ER and associated ribosomes (R).

Figure 6. Part of an interphase nucleolus showing all three components of the nucleolus: pars granulose (Gr), pars fibrosa (Fib), and proteinaceous matrix (PM). Arrow points to a 60 nm nucleolar fibril in the pars fibrosa

Figure 7. Two microbodies (Mb) in the cytoplasm of an interphase cell with dense contents. A crystal-line-like structure is present in one of them

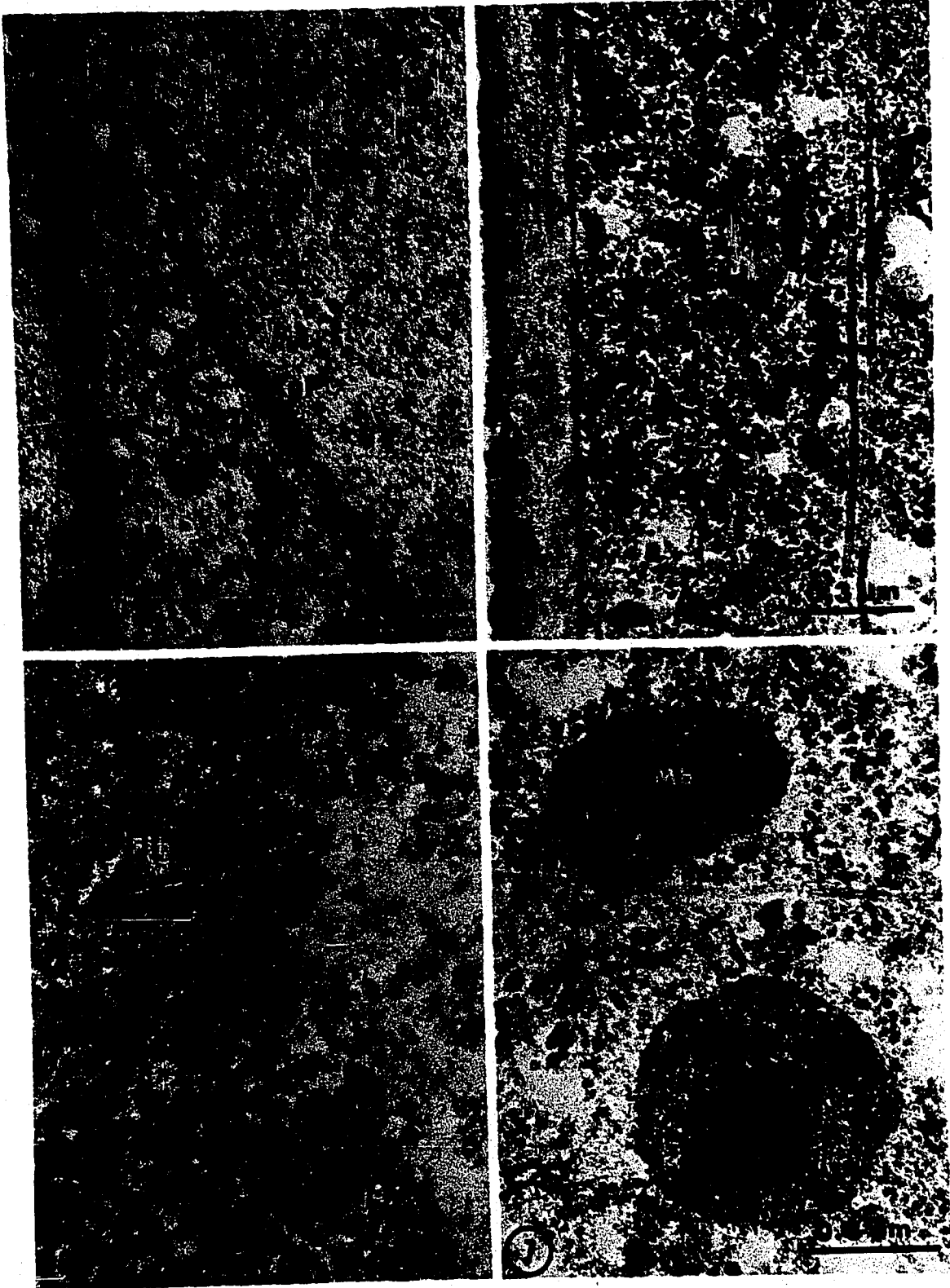


Figure 8. A serial section of an interphase nucleus showing a centriole (C). Inset: enlargement of the centriole

Figure 9. A serial section of the same nucleus as in Figure 8 showing the other centriole at one end of the nucleus. Inset a, b, and c: several adjacent serial sections of the centriole

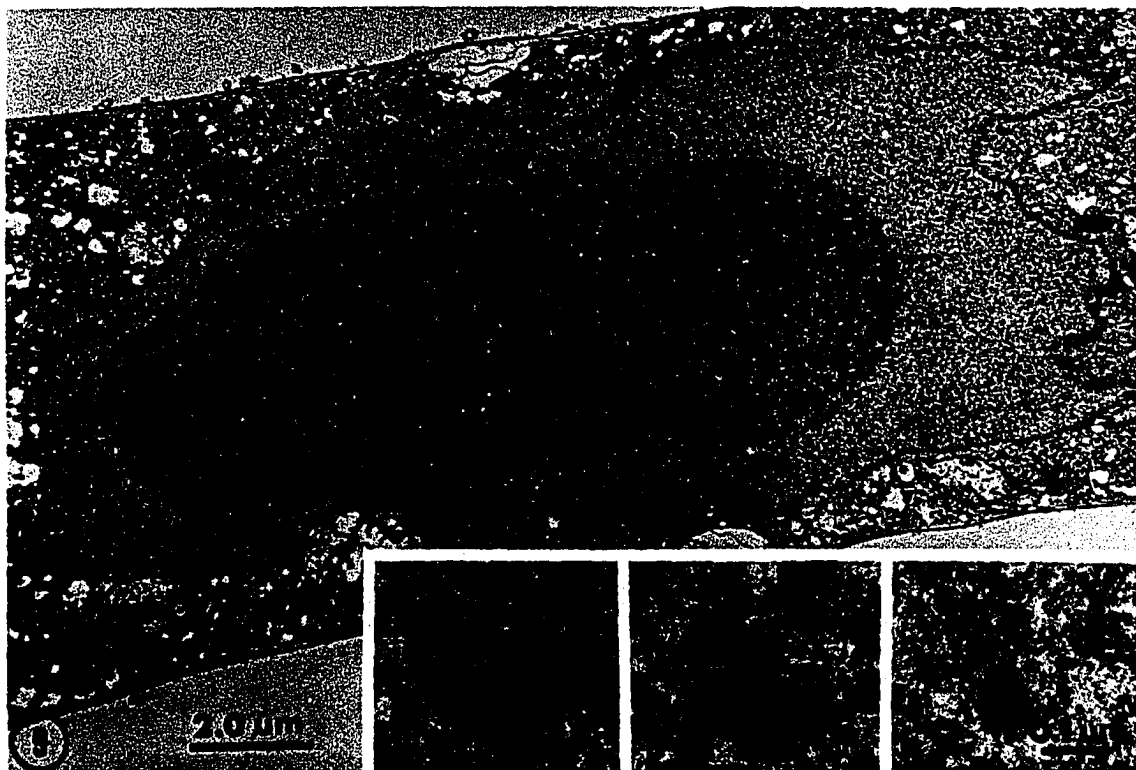
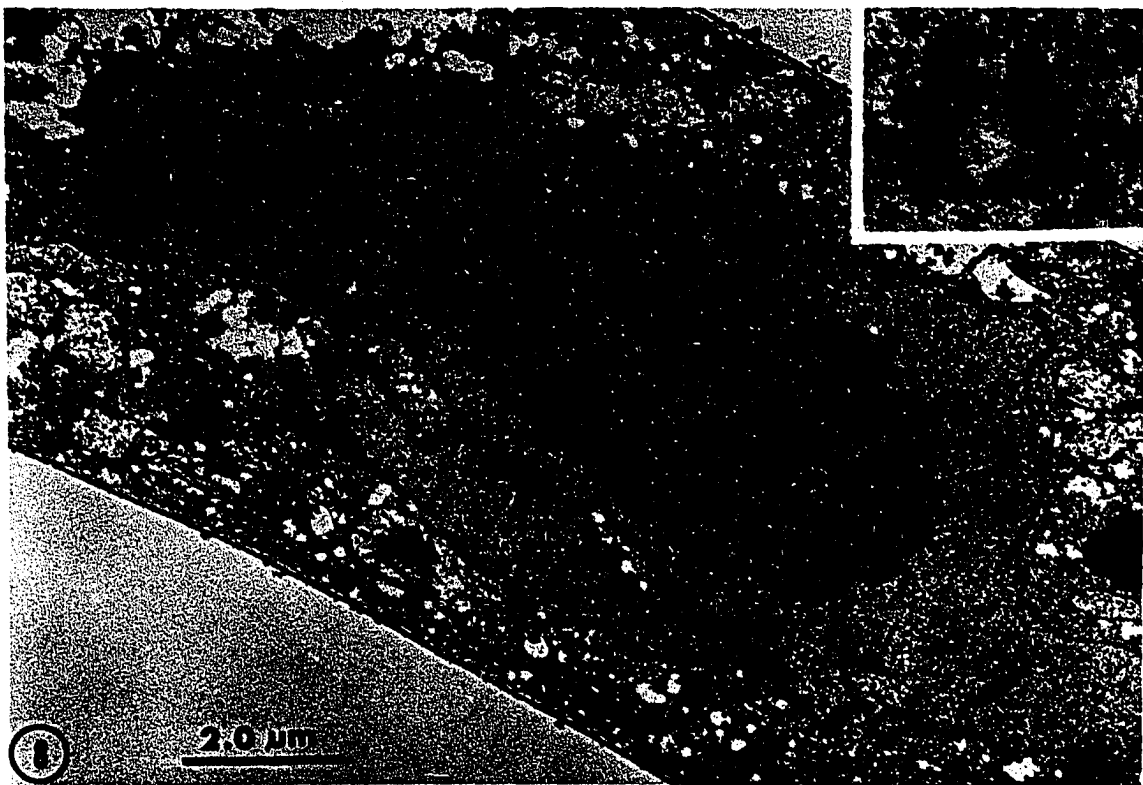


Figure 10. A longitudinal section of the hyphal tip. Small arrows indicate cross sections of microtubules (Mt) and a large arrow points to a longitudinal section of a microtubule. A multiple vesicular body (MVB) is visible

Figure 11. Cytoplasmic microtubules (Mt), aligned with the hyphal axis, and several vesicles (V) found exclusively in the dome

Figure 12. A multiple vesicular body (MVB) with its membrane fused with the plasmalemma

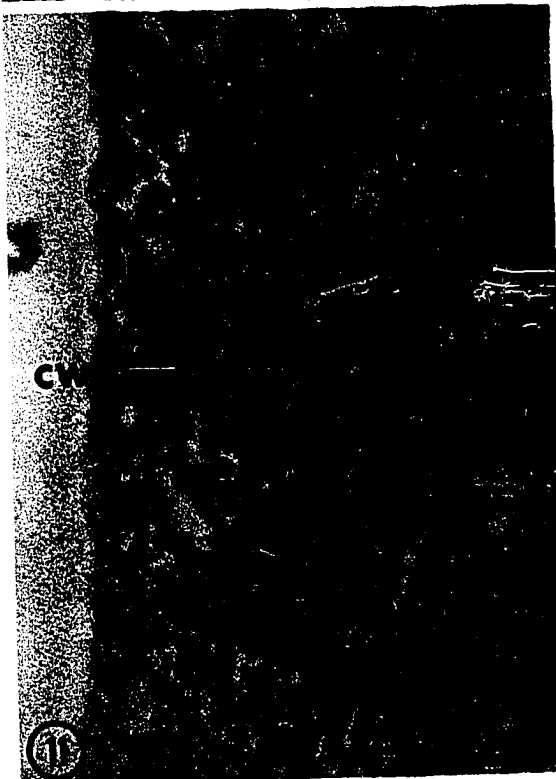
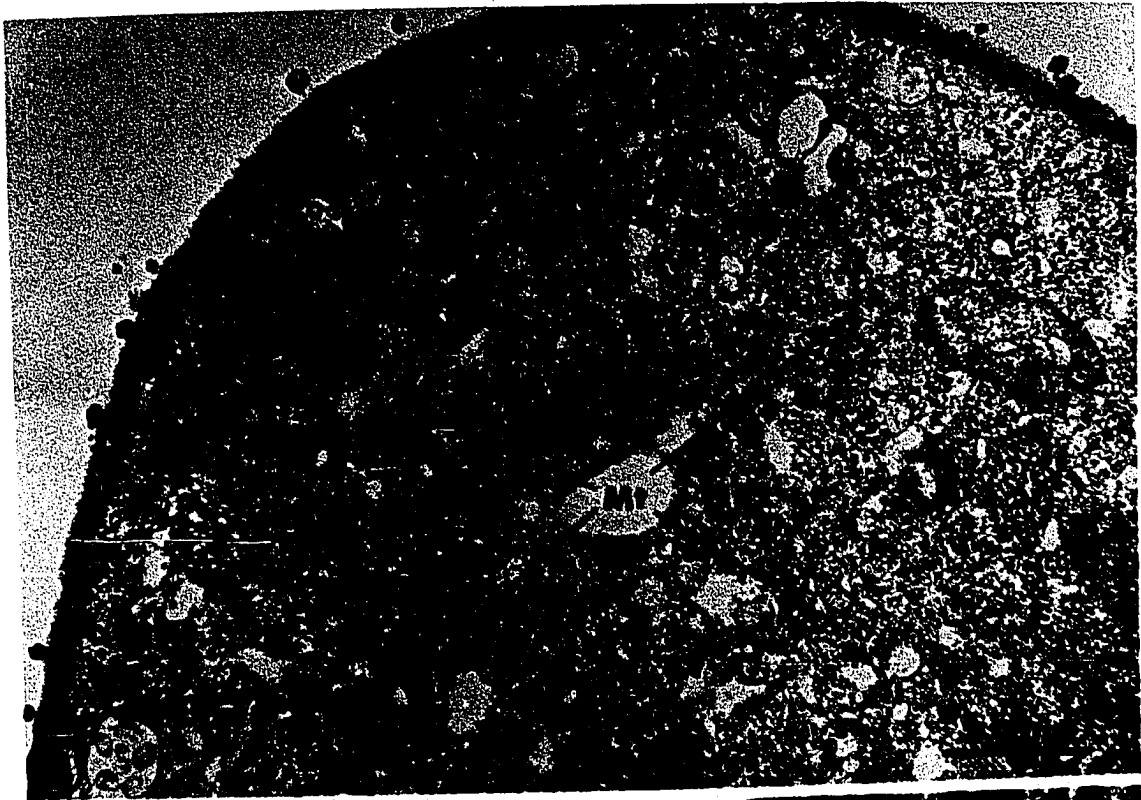


Figure 13. A tangential section of an early prophase nucleus. The nucleolus has dispersed filling up most of the nuclear space; some of the nucleolar granules (NuG) can be found in the nucleoplasm. The distinction between the pars granulosa (Gr) and the pars fibrosa (Fib) becomes barely recognizable. A small centriole (C) in one pole of the nucleus is seen with a few radiating microtubules (Mt). The inset is a light micrograph of the same nucleus embedded, just before thin sectioning

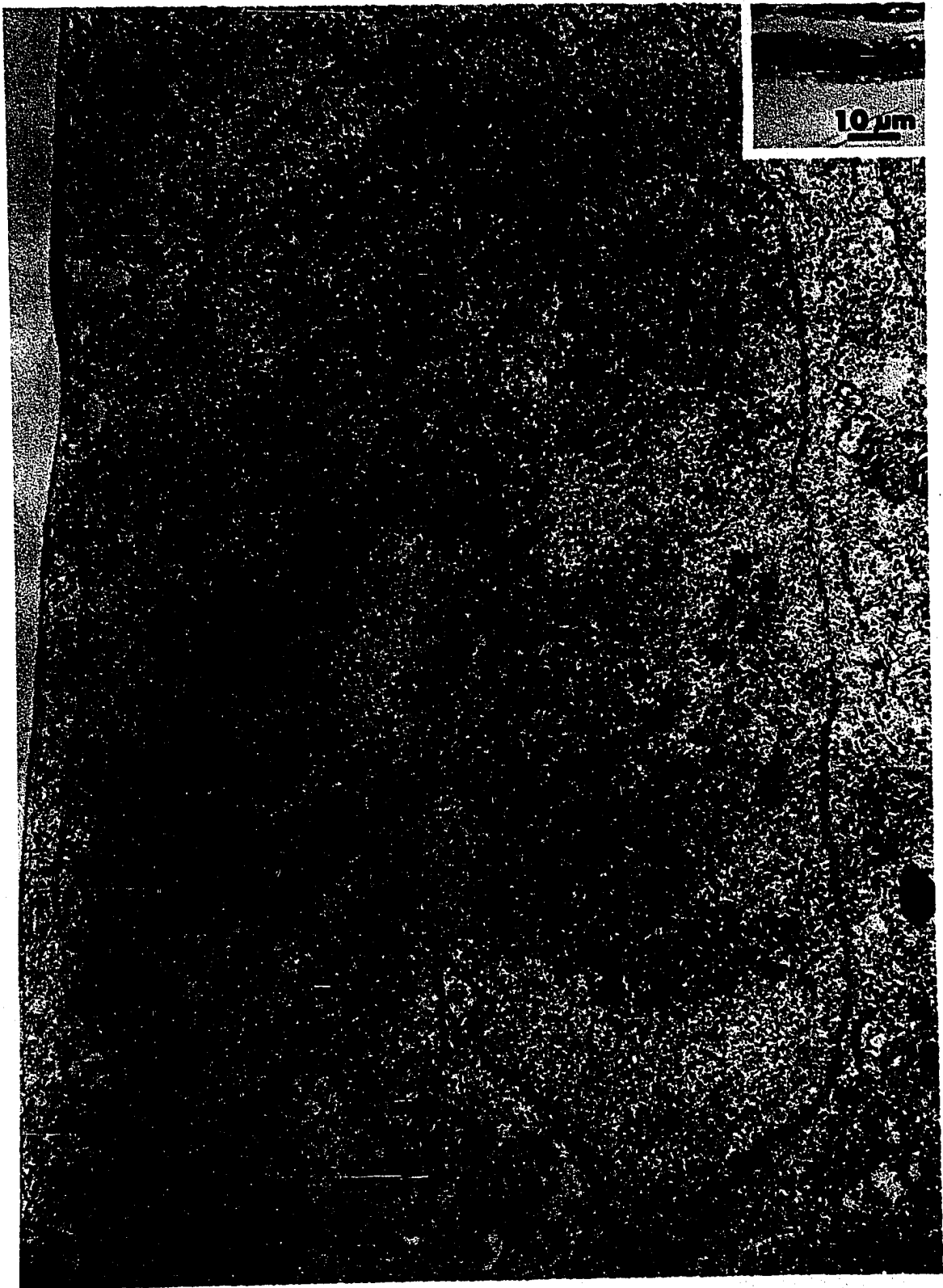


Figure 14. An adjacent serial section of Figure 13 showing the centriole (C) with a small portion of a microtubule (Mt) apparently associated with it. The nuclear plugs (NuP) appear thinner and more diffuse than those in the interphase. Nucleolar granules (NuG) are distributed in the nucleoplasm

Figure 15. A serial section of the same nucleus as in Figure 13 showing the position of the other centriole (C) at the opposite pole of the nucleus. The inset is an enlargement of this centriole showing several microtubules that appear to end in the centriolar wall

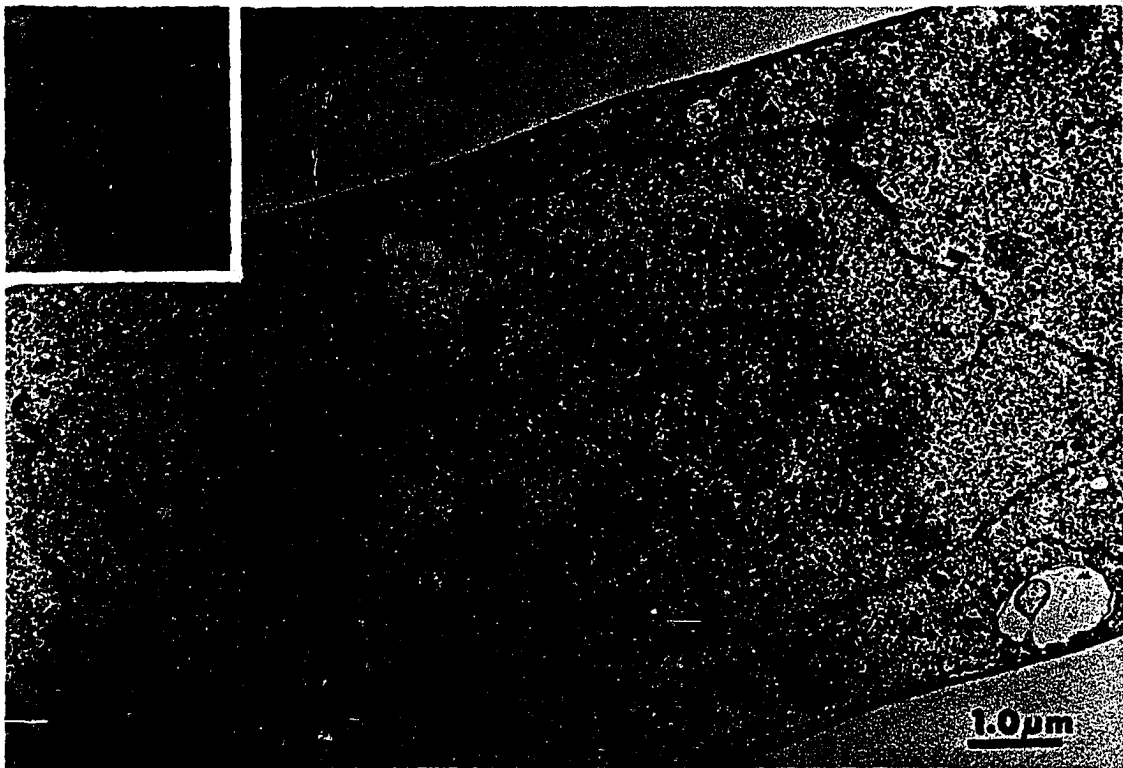
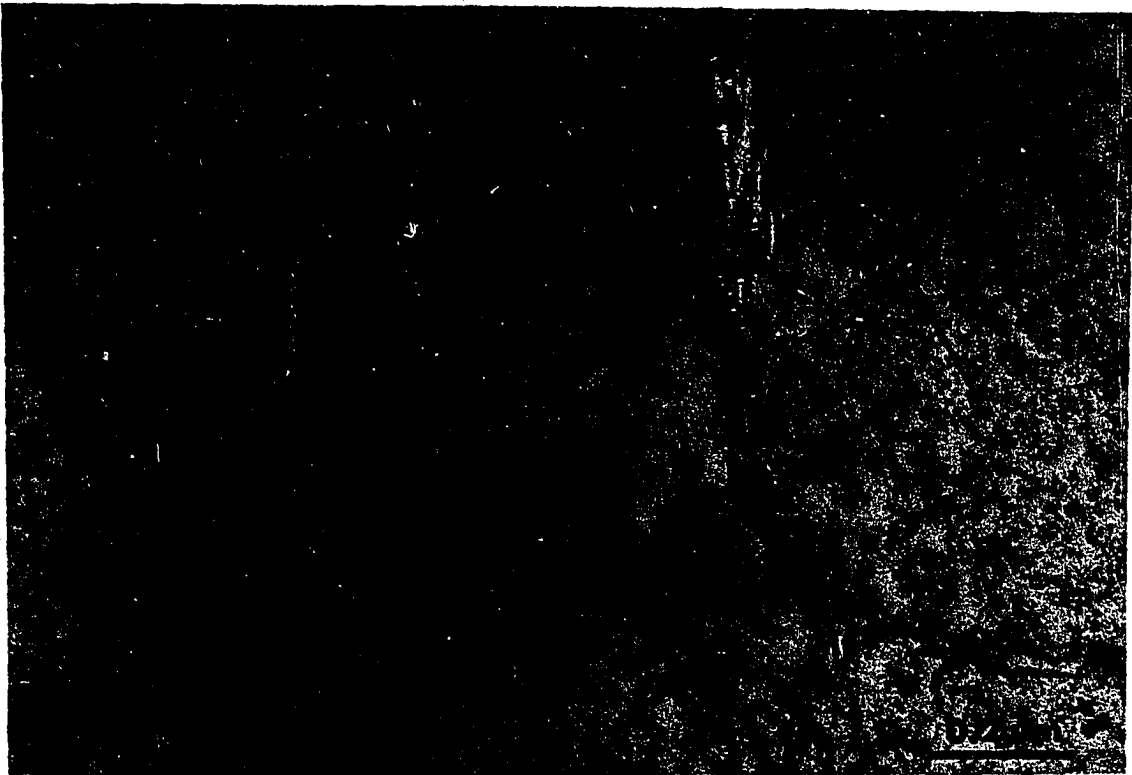


Figure 16. A mid-prophase nucleus. Numerous extranuclear microtubules (Mt) appear tangential to the nucleus near the opposite poles. The pars granulosa has completed its dispersion; the remnants of the nucleolus consist of the pars fibrosa (Fib), small patches of which (NuF) are scattered in nucleoplasm



Figure 17. One pole of the mid-prophase nucleus in Figure 16. Microtubules (Mt) are seen ending at or close to the nuclear envelope. One of them apparently penetrates the nuclear envelope (see large arrow)

Figure 18. The other pole of the mid-prophase nucleus in Figure 16. Microtubules (Mt) are seen ending at or close to the nuclear envelope. A large arrow points to a short piece of microtubule in the nucleoplasm

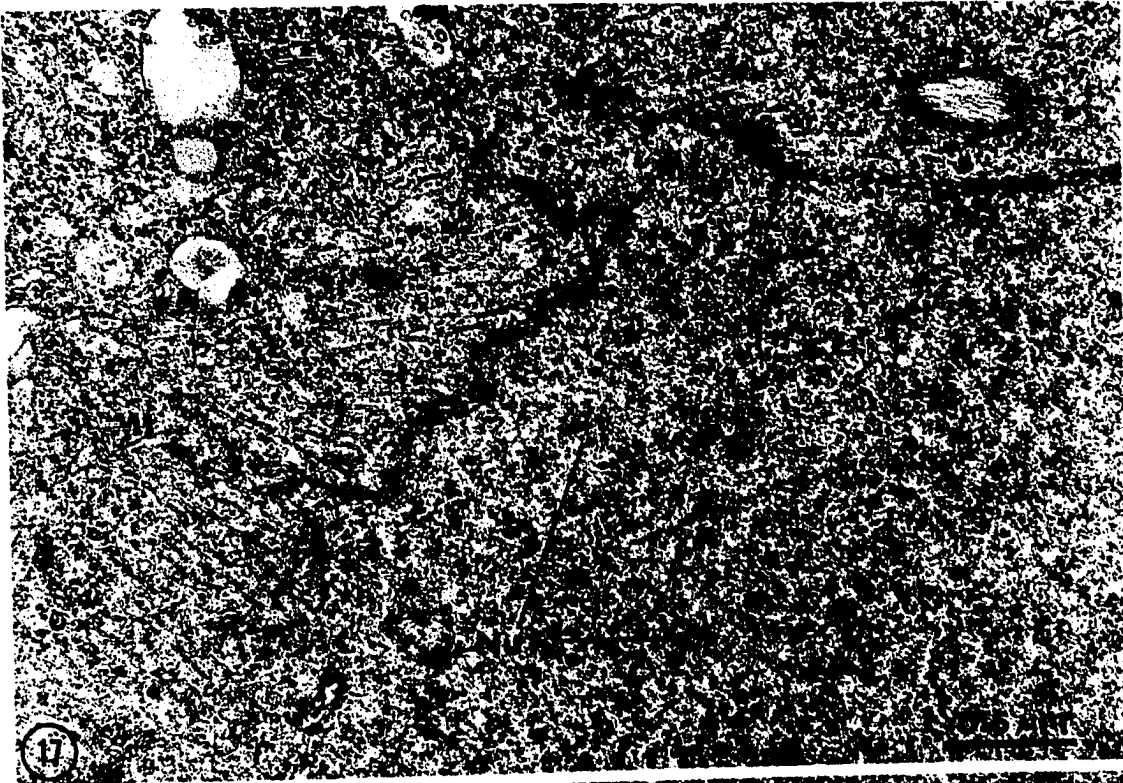


Figure 19. Nuclear envelope breakdown is seen at one pole of this late prophase nucleus

Figure 20. An adjacent serial section of the same nucleus as Figure 19 showing a solitary centriole (C) associated with a fragment of the nuclear envelope (NEF) (see arrows)

Figure 21. Part of the residual pars fibrosa in the square of Figure 19. Arrows suggest 6-8 nm nucleolar fibrils

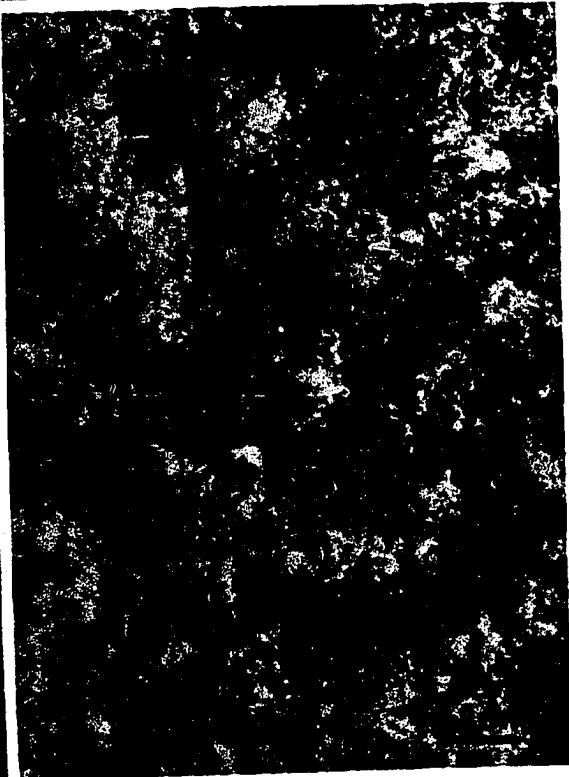
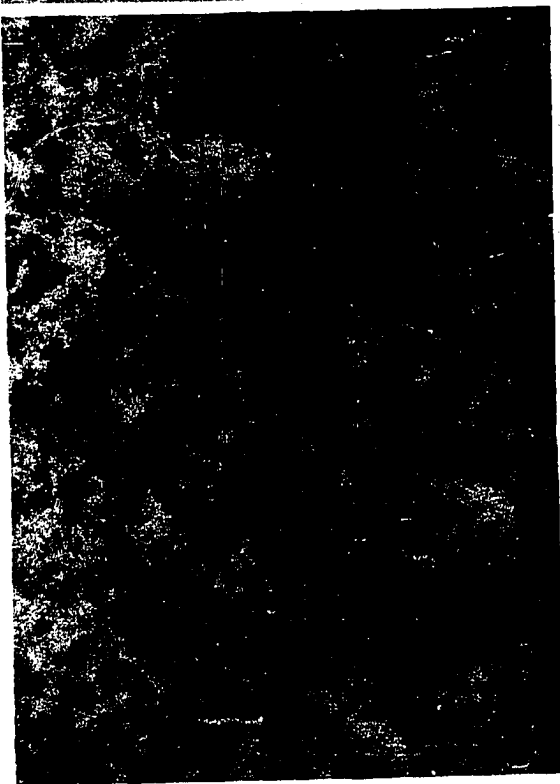
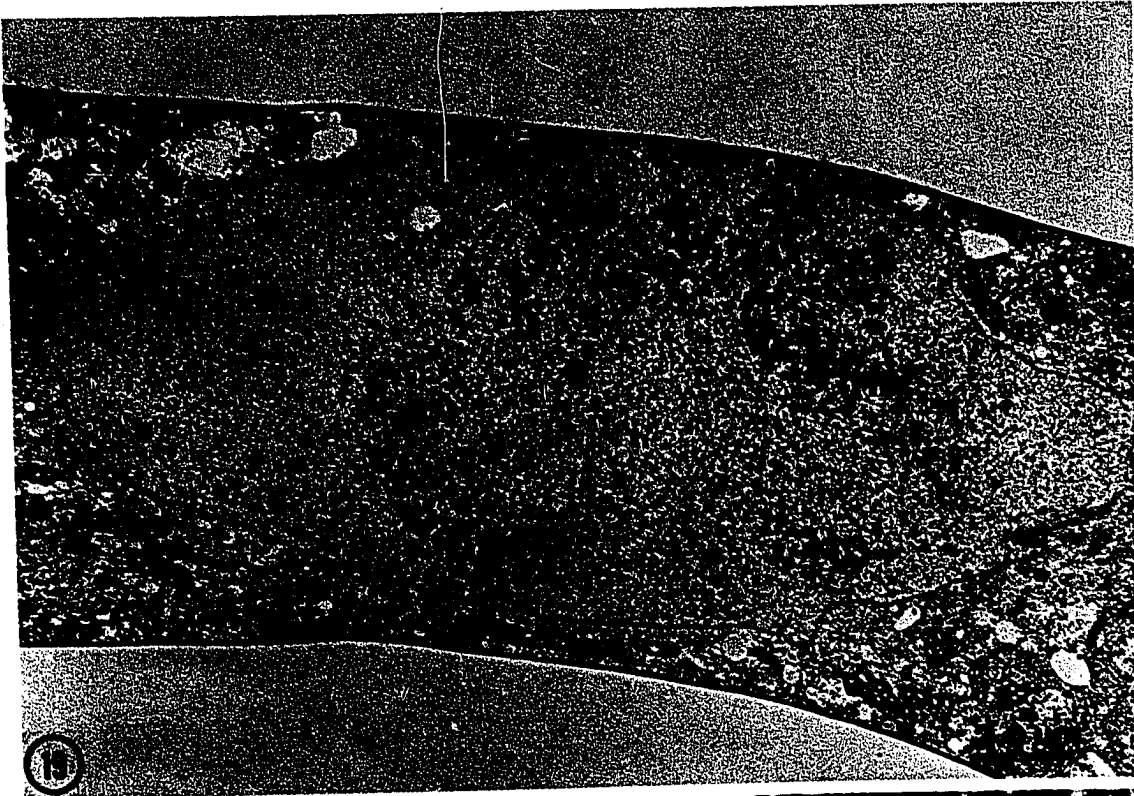


Figure 22. At early prometaphase the nuclear envelope has completely fragmented into many small vesicles and short cisternae (NEF) forming a sphere around the nucleoplasm. Chromosomes (Ch) and spindle microtubules (SMT) become recognizable among the nucleolar materials (NuG) which gradually increase in amount. The light micrograph inset is the same nucleus before thin sectioning

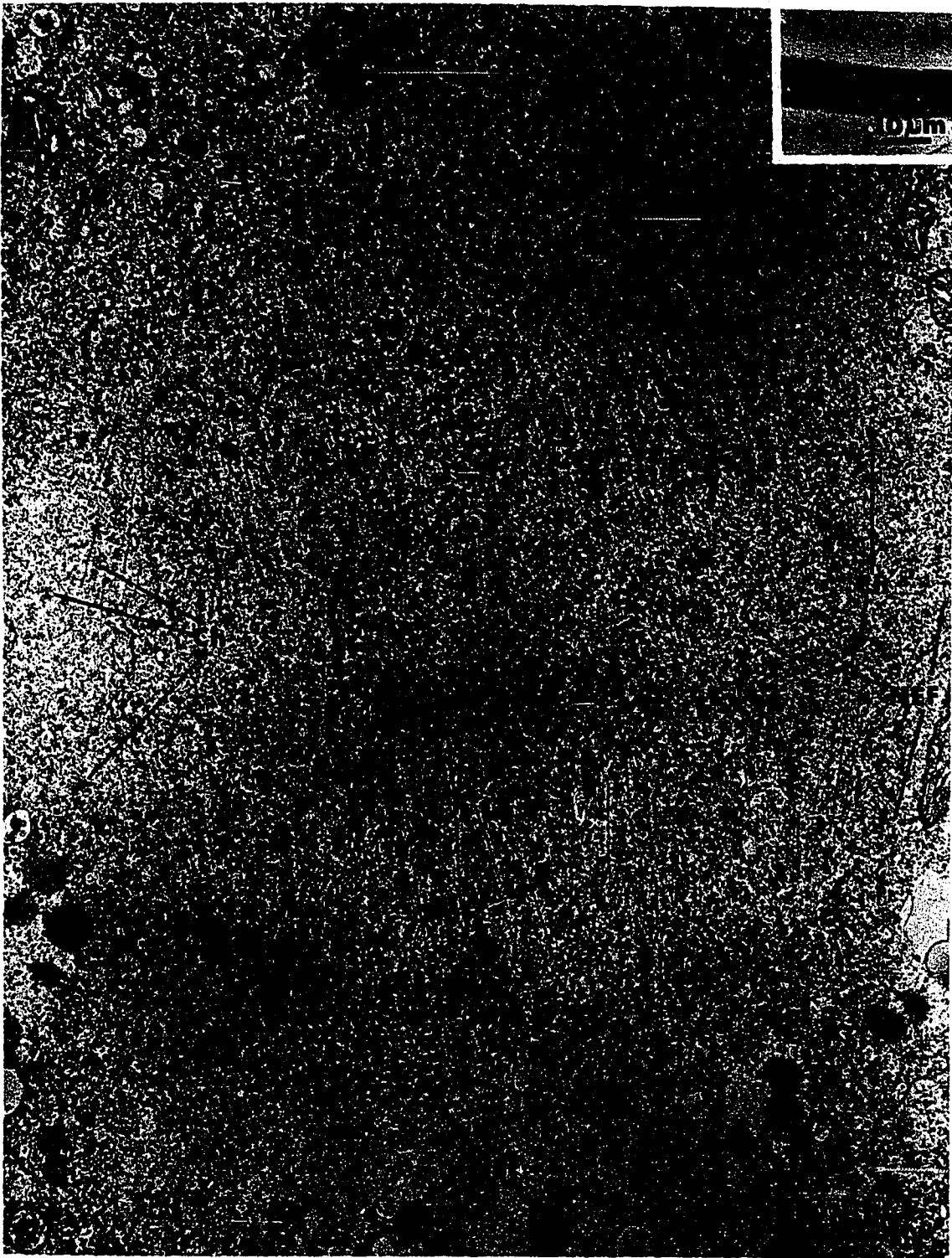


Figure 23. A serial section of the same nucleus as in Figure 22 showing the centriole (C) to one side of the median spindle axis

Figure 24. A high magnification of the centriole in Figure 23. A central hub extending four-fifths the length of the centriole is seen in the center of the centriole. Note microtubule extending from centriole through a gap in the nuclear envelope (arrow)

Figure 25. A serial section of the same centriole showing the tubular substructure of the centriole

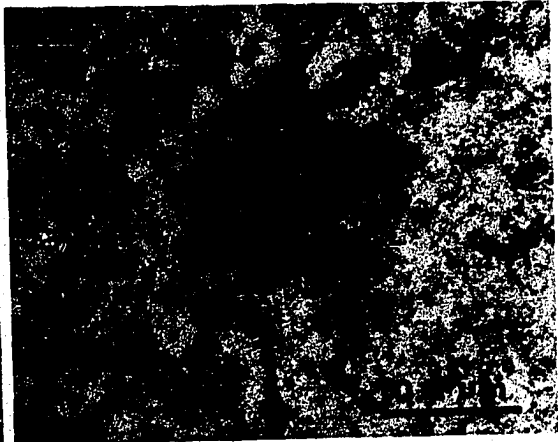
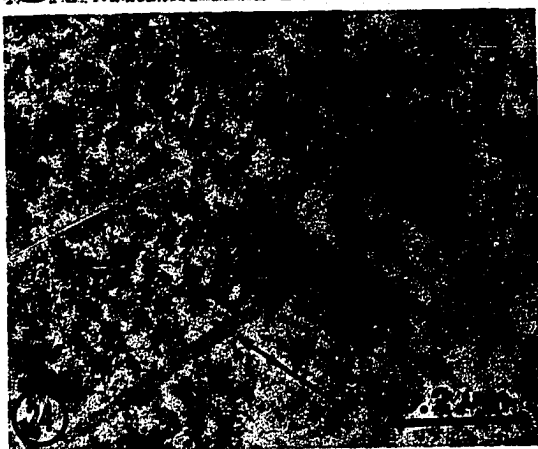
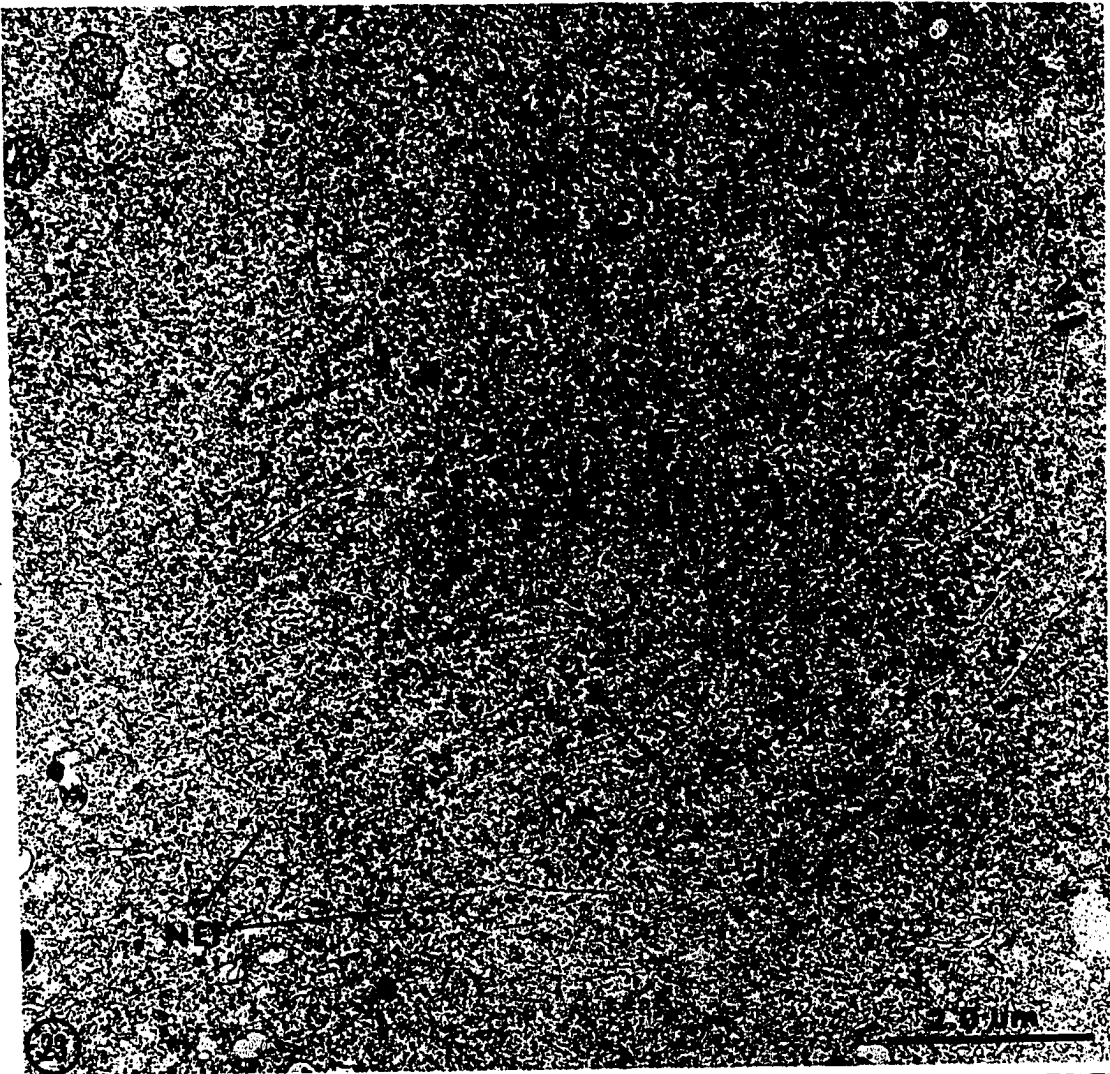


Figure 26. At late prometaphase the nuclear envelope fragments reassociate with each other forming many discontinuous long cisternae (NE). The light micrograph inset is the same nucleus ready for sectioning showing the beginning or organization of nucleolar materials into two separate groups



Figure 27. Early metaphase nucleus. Chromosomes (Ch) have become compact and have moved to the equatorial region. Appearing within the reformed nuclear envelope (NE) is an increasing amount of the nucleolar materials (NuG). The inset is the corresponding light micrograph of the same embedded nucleus prior to sectioning

Figures 28, 29, and 30. Three serial sections of two paired centrioles (C_1 and C_2) found in adjacent serial sections of the same nucleus in Figure 27

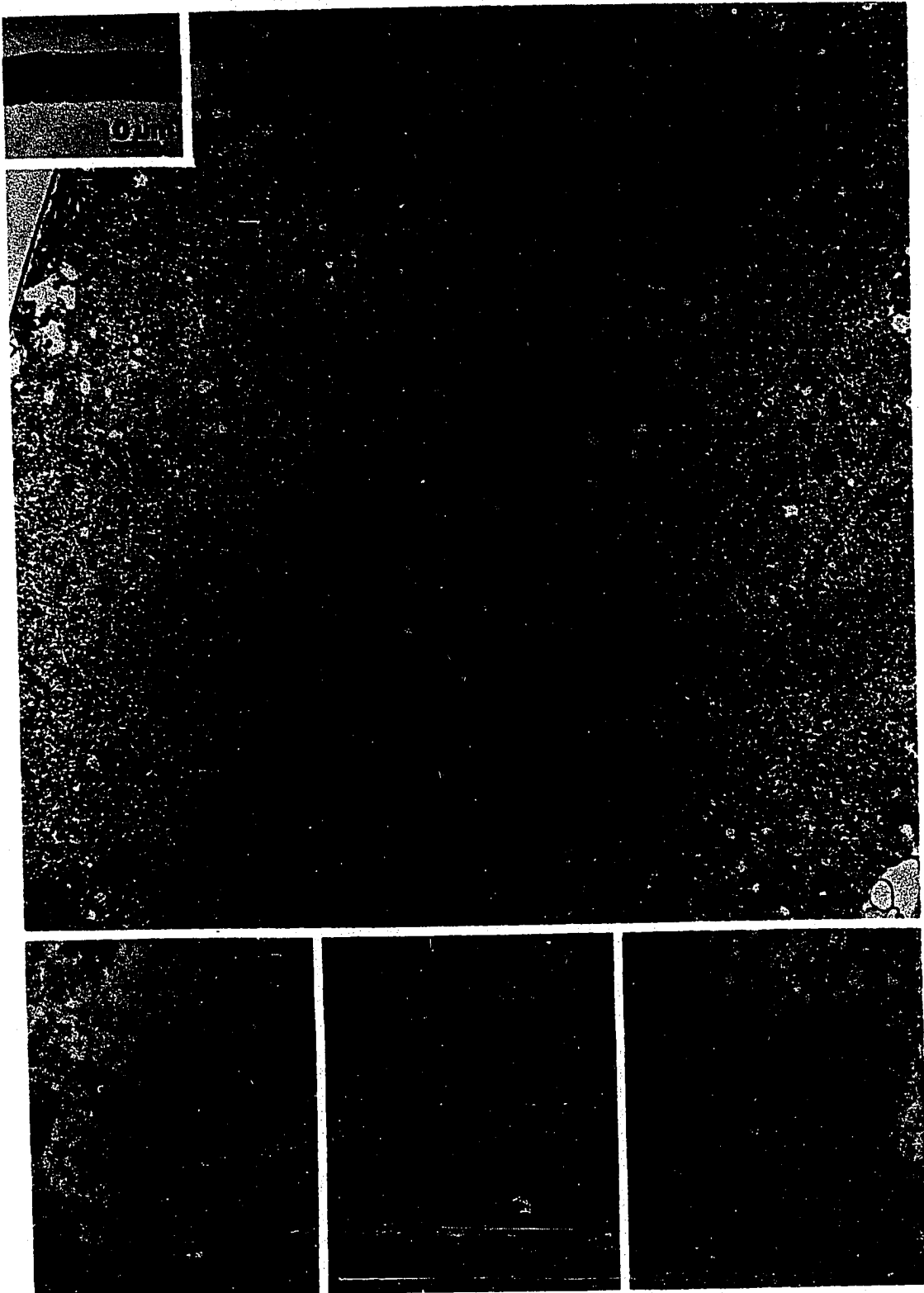


Figure 31. Part of the early metaphase nucleus (the rectangle in Figure 27) showing the reformed nuclear envelope (NE) and the abundant ER around the nucleus. A possible ER-nuclear envelope association is indicated by an arrow

Figure 32. Part of an early metaphase nucleus (the square in Figure 27) showing chromosomes distributed among the nucleolar materials. A 15-20 nm nucleolar granule is indicated by an arrow

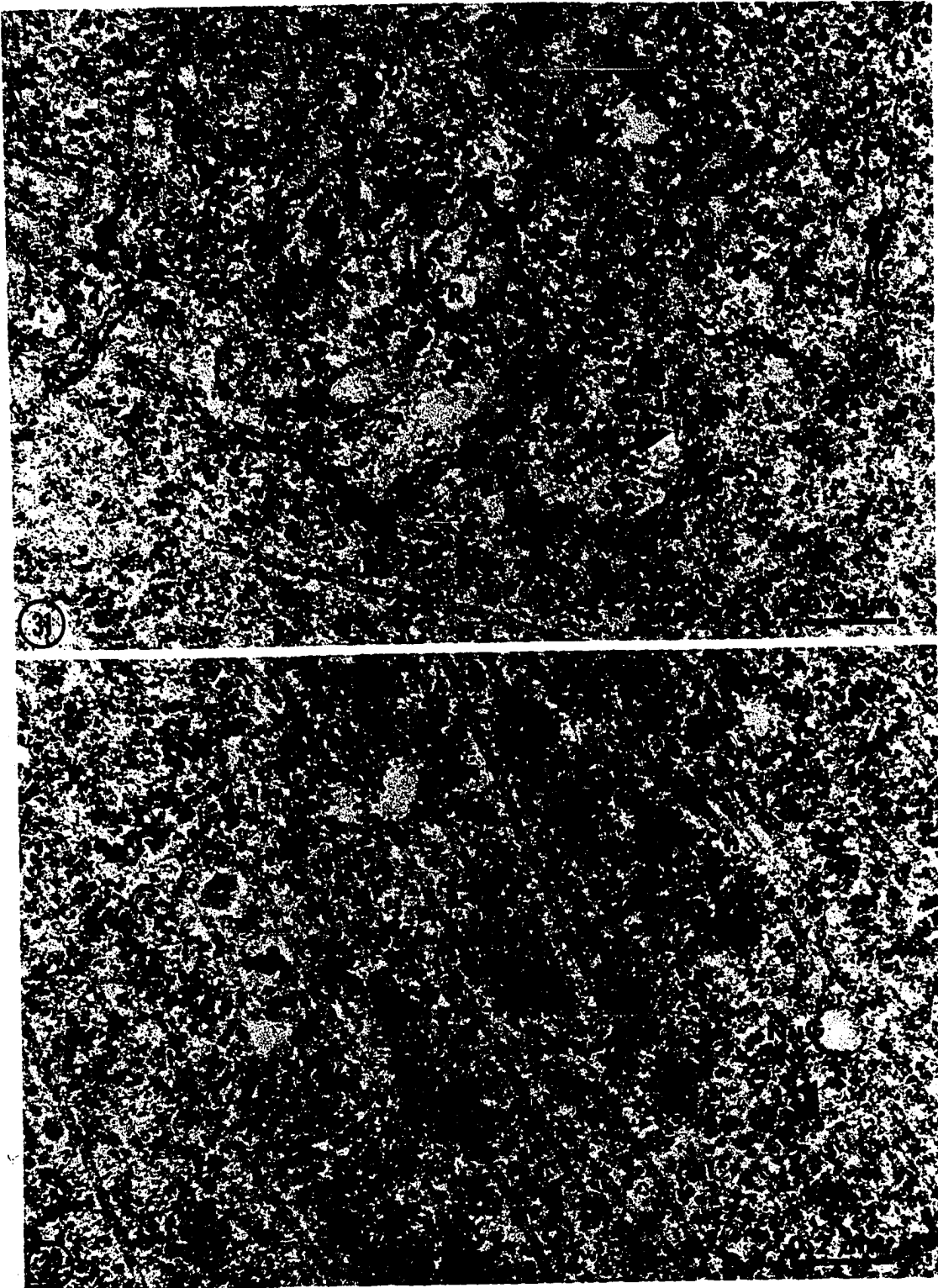


Figure 33. The rotating stage between early metaphase and late metaphase. One-half of the spindle microtubules and one-half of the metaphase plate (MP_1) have completed the 90° rotation; the spindle microtubules are oriented normal to the hyphal long axis. The remaining spindle microtubules and one-half of the metaphase plate (MP_2) retain their original position with the spindle axis parallel to the hyphal long axis. The inset is a light micrograph of the same nucleus before sectioning



Figure 34. A late metaphase nucleus showing an orderly equatorial alignment of chromosomes (Ch). The approximate positions of two pairs of orthogonally-arrayed centrioles, found in the adjacent serial sections, are indicated by arrows. The insets show high magnifications of the two pairs of centrioles



Figure 35. A metaphase chromosomes (Ch). Only one of the two cone-shaped chromatids and the diffuse chromatin between them are shown. The relatively light tip of the chromatid is one-half of the already divided kinetochore (K) with which a single microtubule (ChF) is associated. The arrows point to 3-4 nm chromosomal fibrils (ChrF).

Figure 36. A metaphase chromosome (Ch) showing microtubule (ChF) appearing to penetrate the kinetochore (K). The small arrow points to a 3-4 nm chromosomal fibril (ChrF) in the kinetochore

Figure 37. An anaphase chromosome (Ch) showing a relatively light kinetochore (K) and an associated microtubule

Figure 38. An anaphase chromosome (Ch) showing a part of the microtubule (ChF) apparently within the kinetochore (K)

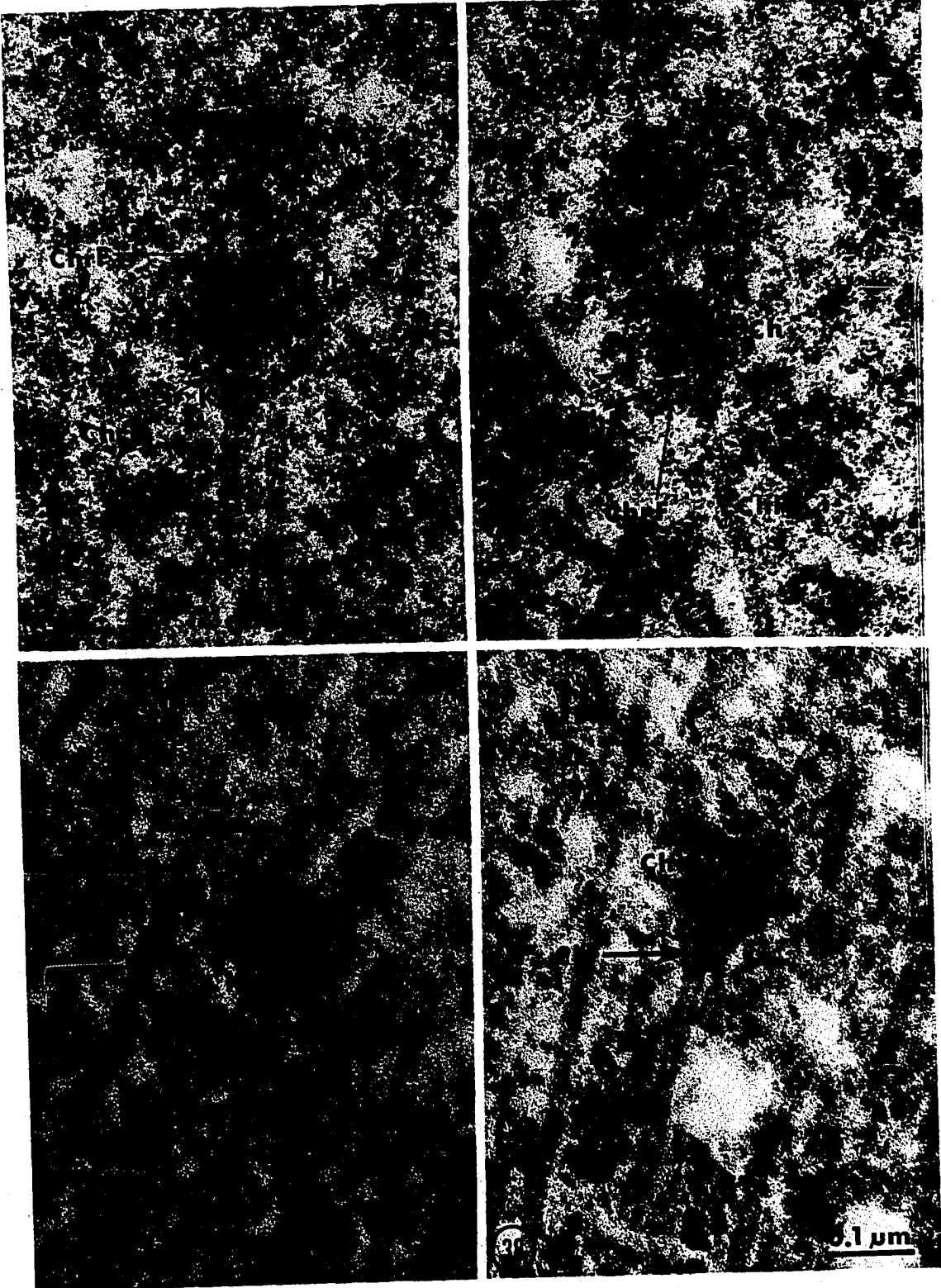
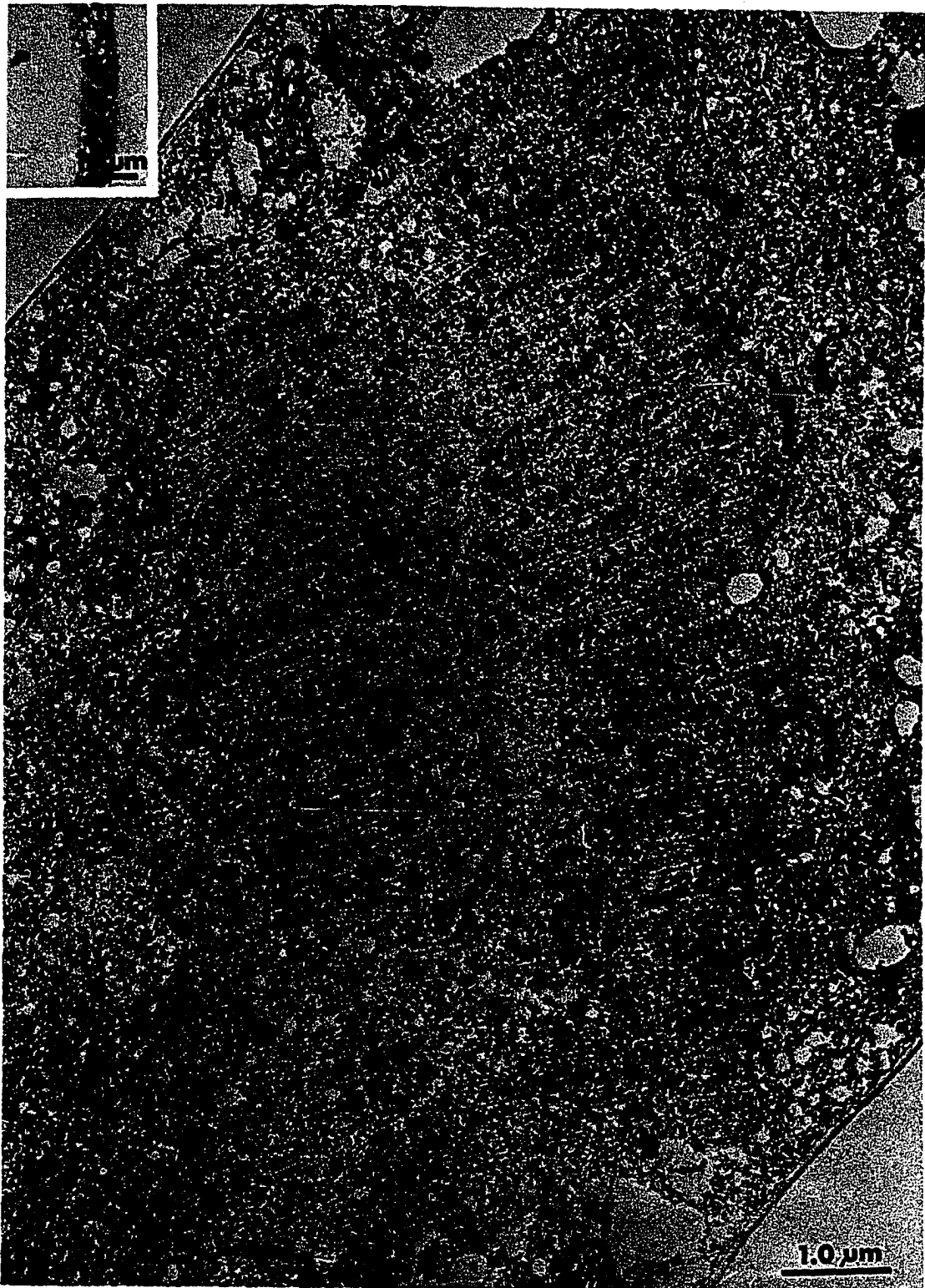


Figure 39. An early anaphase nucleus. The separation of daughter chromosomes (Ch) makes it possible to distinguish chromosome fibers from continuous fibers (CF). The arrow points at a microtubule running $\frac{4}{5}$ the length of the spindle axis. The inset is a light micrograph of the same nucleus prior to sectioning



Figures 40 and 41. Shown here are two sections from an extensive set of serial sections of the same nucleus as in Figure 39

Figure 40. Paired centrioles (C) are found at one pole of the nucleus. The inset is a high magnification of these centrioles

Figure 41. Another centriole (C) is found at the opposite pole of this nucleus. No mate to this centriole was noted, but the possibility of one is not ruled out. The inset is a high magnification of this centriole

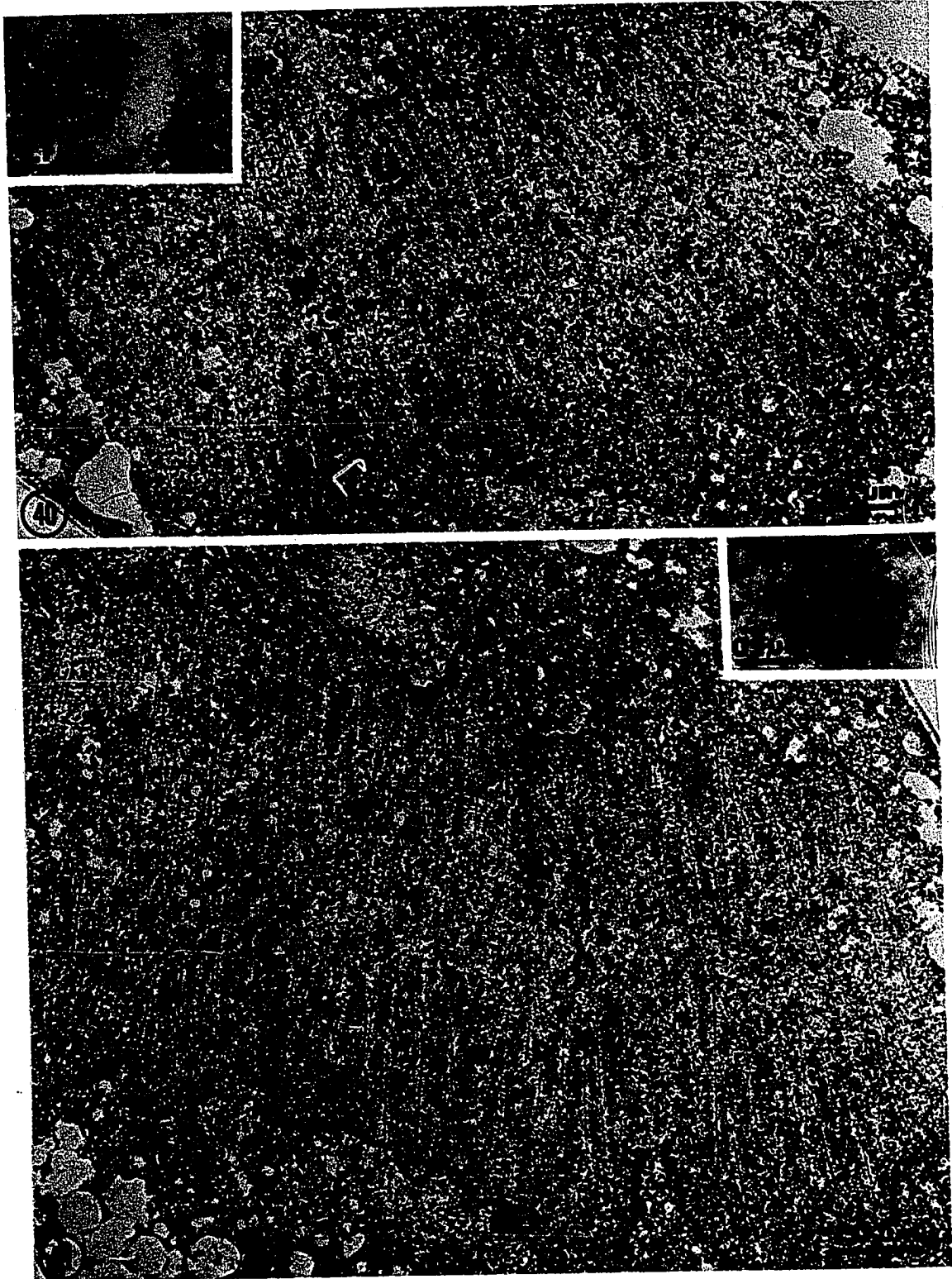


Figure 42. A late anaphase nucleus. The increasing length of the continuous fibers (CF) may be responsible for the final separation of daughter chromosomes and with their associated nucleolar dense bodies. The inset is a light micrograph of the same nucleus before sectioning



Figure 43. The differentiation between the nucleoplasm (Nm) and the nucleolus (Nu) appears in telophase nuclei. Continuous fibers (CF) remain only around each gap in the nuclear envelope. The inset is a light micrograph of the cell before sectioning

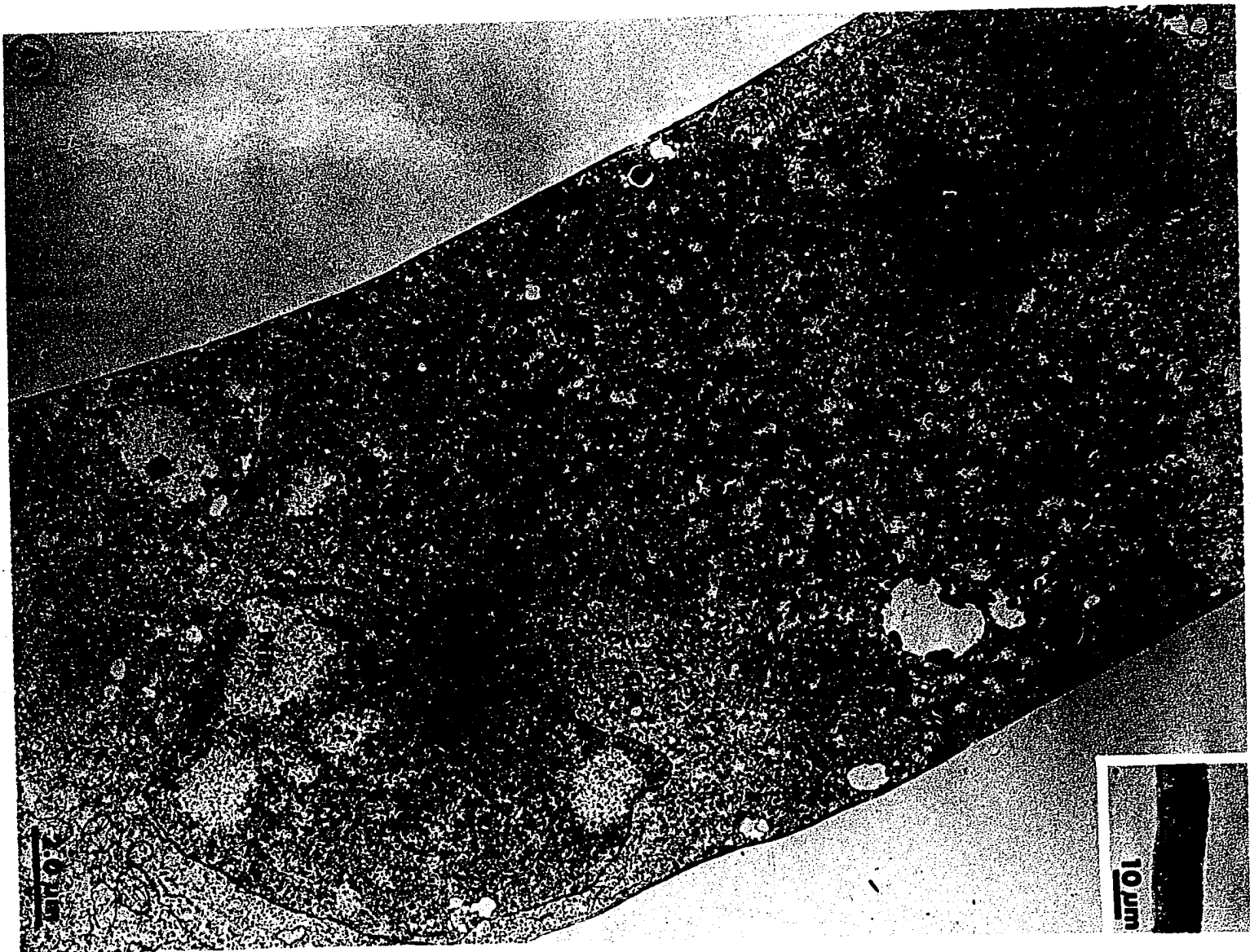


Figure 44. One of the two nuclei in Figure 43. Nuclear pores with nuclear plugs (NuP) are evident

